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(54) Title: DETECTION AND ALTERATION OF MHC CLASS II PRESENTATION PHENOTYPES

(57) Abstract: The present invention relates to the discovery of a group of abnormal MHC class II presentation phenotypes, which are predictive of penetrance of a disorder characterized by abnormal MHC class II antigen presentation, e.g., in comparison to cells (even genetically identical cells) from individuals not afflicted with the disorder. The invention provides methods for detecting an individual's predisposition to develop a disorder associated with abnormal MHC class II presentation. The present invention is also directed to a method for treating a disease or disorder associated with abnormal MHC class II presentation (e.g., Type I diabetes) by effectively altering MHC class II presentation of an antigen presenting cell.

DETECTION AND ALTERATION OF MHC CLASS II PRESENTATION PHENOTYPES

Field of the Invention

The present invention is in the fields of cellular biology and immunobiology. The present invention is directed to novel methods for assaying and altering cellular MHC class II presentation.

Background of the Invention

Autoimmune diseases are clinically distinct but cluster in families, and the co-existence of two or more diseases in the same patient is not uncommon (Becker, 1999; Bias, 1999; Lin, 1998; Wu, 1996). Published genetic studies, even in the human, confirm the clustering of similar genome-wide genetic loci, supporting a hypothesis that clinically distinct autoimmune phenotypes are controlled by common sets of susceptibility genes (Becker, 1998). This hypothesis has recently been referred to as the "Common Gene Hypothesis."

Autoimmune diseases also share other common traits. For example, all autoimmune diseases show incomplete penetrance between identical twins (Bellamy, 1992; Silman, 1993), suggesting some critical epigenetic factor(s) influencing disease penetrance. Despite this, genetic studies still support the commonality of all autoimmune diseases with linkage to the highest risk region; the Major Histocompatability Complex class II region. Autoimmune diseases with suspected MHC class II linkage include: Alopecia Areata, Ankylosing Spondylitis, Antiphospholipid Syndrome, Autoimmune Addison's Disease, Autoimmune Dermatitis, Autoimmune Hemolytic Anemia, Autoimmune Hepatitis, Behçet's Disease, Bullous Pemphigoid, Cardiomyopathy, Celiac Sprue-Dermatitis, Chronic Fatigue Immune Dysfunction Syndrome (CFIDS), Chronic Inflammatory Demyelinating Polyneuropathy, Churg-Strauss Syndrome,

Cicatricial Pemphigoid, CREST Syndrome, Cold Agglutinin Disease, Crohn's Disease, Discoid Lupus, Essential Mixed Cryoglobulinemia, Fibromyalgia-Fibromyositis, Graves' Disease, Guillain-Barré, Hashimoto's Thyroiditis, Idiopathic Pulmonary Fibrosis, Idiopathic Thrombocytopenia Purpura (ITP), IgA Nephropathy, Insulin Dependent Diabetes Mellitis (IDDM), Juvenile Arthritis, Lichen Planus, Lupus, Ménière's Disease, Mixed Connective Tissue Disease, Multiple Sclerosis, Myasthenia Gravis, Pemphigus Vulgaris, Pernicious Anemia, Polyarteritis Nodosa, Polychondritis, Polyglandular Syndromes, Polymyalgia Rheumatica, Polymyositis and Dermatomyositis, Primary Agammaglobulinemia, Primary Biliary Cirrhosis, Psoriasis, Raynaud's Phenomenon, Reiter's Syndrome, Rheumatic Fever, Rheumatoid Arthritis, Sarcoidosis, Scleroderma, Sjögren's Syndrome, Stiff-Man Syndrome, Takayasu Arteritis, Temporal Arteritis/Giant Cell Arteritis, Ulcerative Colitis, Uveitis, Vasculitis, Vitiligo, and Wegener's Granulomatosis.

Proteins encoded by genes of the Major Histocompatability Complex (MHC) in higher vertebrates (e.g., mammals) play an essential role in regulating the immune system. MHC molecules are derived from a set of linked genetic loci and are the most polymorphic known in higher vertebrates, with any given species exhibiting an extraordinarily large number of alleles for each MHC locus. MHC proteins, expressed in a vertebrate cell, form complexes with antigenic peptides and are displayed on the surface of the cell where they are recognized by T cells. There are two classes of MHC proteins, known as MHC class I and class II. They are similar in that they both form a binding groove for complexing with antigen peptides and both form antigen peptide complexes for presentation of an antigen in a conformation recognizable by specific T cells; however, the class I and class II proteins perform independent and distinct functions in regulating immune responses to antigenic proteins.

MHC class I proteins are expressed in all nucleated cells of higher vertebrates. MHC class I molecules bind peptides derived from endogenous antigens (e.g., normal, "self" cellular proteins, or viral or bacterial proteins produced within an infected cell), which have been processed within the cytoplasm of the cell (the cytosolic pathway). MHC class I antigen complexes, properly displayed on the surface of the cell are recognized by cytotoxic T cells (specifically $CD8^+$ T_C cells). Presentation of an endogenous or "self" peptide by the MHC class I antigen complex is protective; under normal circumstances, cytotoxic T cells that would otherwise recognize the surface complex and attack the presenting cell have been eliminated (deleted) from the immune system repertoire. Presentation of "foreign" (exogenous or "non-self") peptides by the MHC class I antigen complex elicits cytotoxic T cell attack and cytolytic destruction of the infected or diseased cell. Thus, MHC class I antigen complexes either mark

the cell as a normal endogenous cell, which elicits no immune response, or mark the cell as an infected cell (e.g., as in the case of a virus-infected cell, exhibiting intracellularly processed viral peptide in the surface MHC class I antigen complex) or a transformed cell (e.g., such as a malignant cell), which elicits attack on the cell by cytotoxic T cells.

MHC class II proteins are expressed in a subset of nucleated vertebrate cells, conventionally referred to as Antigen Presenting Cells, or "APCs". The MHC class II molecule is a heterodimer formed from α and β subunits. MHC class II molecules bind peptides derived from exogenous antigens, which are internalized by phagocytosis or endocytosis and processed within the endocytic/lysosomal pathway. MHC class II antigen complexes, properly displayed on the surface of an APC are recognized by helper T cells (specifically $CD4^+$ T_H cells). Helper T cell recognition results in release of lymphokines and T-dependent activation of B cells, which, in turn, lead to activation of macrophages and release of antibodies from B cells, leading to the killing or elimination of invading microorganisms. The immune recognition events mediated by MHC class II antigen complexes are a primary defense to invading microorganisms (e.g., bacteria, parasites) or foreign substances (e.g., haptens, transplant tissues) introduced to the cells of the immune system via the circulatory or lymph systems.

Because of the complex interaction of genetic and epigenetic factors involved in the etiology of autoimmune disease, accurate and sensitive diagnostic tests for autoimmune disease are lacking in the art. Diagnostic screening is further complicated, yet more imperative, because discernible symptoms of autoimmune disease often do not manifest until late in the progression of the disease. It is critically important to be able to identify individuals at risk for autoimmune disease early on. Low concordance and unpredictable penetrance of the disease renders genetic testing of limited value. Therefore there is a need in the art to discover and to develop a method for identifying afflicted and at-risk individuals quickly and early in the onset of autoimmune disease.

In addition, because the ultimate causes and progression of autoimmune disease are currently not understood, treatment strategies typically focus on remediating the symptoms of the disease. There is a need in the art, therefore, to understand the ultimate causes of autoimmune disease and to discover and to develop effective therapeutic methods useful for directly treating and preventing autoimmune disease more effectively and more permanently.

Summary of the Invention

The present invention relates to the discovery of a group of abnormal MHC class II presentation phenotypes which are indicative of autoimmune disease penetrance and thus are

useful in early diagnosis of autoimmune disease such as Type I diabetes and other class II-linked diseases. This invention provides a novel test for an individual's predisposition to, or current affliction with, a disease or disorder associated with abnormal MHC class II presentation (e.g., Type I diabetes) based upon the detection of abnormal MHC class II presentation. This represents the first report of a significant epigenetic component (i.e., nonallelic influences on a phenotype) in the onset and progression of an autoimmune disease. The present invention is also directed to a method for treating a disease or disorder associated with abnormal MHC class II presentation by altering MHC class II presentation of a cell.

Disclosed herein for the first time is the discovery that in certain autoimmune diseases such as Type I diabetes, diabetic cells are characterized by an abnormal cellular phenotype directed to MHC class II presentation. Specifically, these MHC class II-related phenotypes are abnormal in diabetic twin cells compared to APCs of a non-diabetic identical twin and to non-diabetic control cells. Moreover, these abnormal phenotypes are exclusive to diabetic APCs in discordant identical twin studies. This discovery refutes current teachings concerning MHC class II allelic product influences as the sole cause of disease by diverse mechanisms and favors of MHC class II epigenetic factors.

Also disclosed for the first time is the discovery that specific isoforms of the invariant (Ii) chain molecule that function in routing MHC class II molecules to the Golgi complex and endosomal compartments have profound effects on MHC class II presentation phenotypes, affecting downstream MHC class II glycosylation, endosomal degradation, peptide loading, and surface display. Specifically, the N-terminal endoplasmic reticulum (ER) retention peptide contained within an alternate N-terminal translational start site region, which exists in some Ii chain isoforms but is absent in others, is a key signal peptide for proper MHC class II presentation. Absence of the ER retention signal peptide, known to affect MHC class II transport to the endocytic pathway, results in rapid transport to the cell surface, altering transport through the Golgi complex or bypassing the Golgi complex altogether, and produces abnormal MHC class II presentation phenotypes.

It is therefore an object of the present invention to provide an assay for detecting a disease or disorder associated with abnormal MHC class II antigen presentation (e.g., Type I diabetes) by detecting an abnormal MHC class II presentation phenotype. Another feature of the invention is the detection and/or alteration of a MHC class II presentation phenotype. An abnormal MHC class II presentation phenotype (i.e., any detectable deviation of MHC class II presentation from that found in normally functioning APCs) signals penetrance of a disease or disorder associated with abnormal MHC class II antigen presentation, and restoring or increasing

normal MHC class II antigen presentation on APCs can reverse the effects of disease penetrance, preferably during early phases of the disease prior to target end organ destruction.

It is a further object of the invention to provide an assay for abnormal MHC class II presentation based upon abnormal invariant chain (Ii chain) trimer formation and/or individual Ii chain isoform expression levels. In one preferred embodiment, the assay is directed to detecting abnormal (e.g., reduced) levels of an Ii chain isomer, wherein the Ii chain isomer possesses an ER retention signal, preferably the 16 amino acid alternate translational start site peptide.

In another preferred embodiment, the assay is directed to detecting abnormal (e.g., reduced) levels of Ii chain trimer, containing at least one Ii-p35 or Ii-p43 isomer. Most preferably, the assay is directed to detecting abnormal (e.g., reduced) levels of Ii-p35 and/or Ii-p43.

It is also an object of the invention to provide a method for altering MHC class II presentation of a cell. The present method for altering MHC class II presentation of a cell may be obtained by altering any one or more of the group of MHC class II phenotypes identified above. In another embodiment, cellular MHC class II presentation is altered by providing the cell with MHC class II molecules which exhibit a different (preferably greater) affinity for Ii chain molecules (preferably the CLIP fragment of the Ii chain) than otherwise possessed by the cell. In another embodiment, the cell is provided with one or more inhibitors of Ii chain degradation enzymes (e.g., endosomal proteases). In a further embodiment, cellular MHC class II presentation is altered by altering MHC class II-Ii phosphorylation within the cell sufficient to affect Ii chain endosomal delivery and/or degradation. Another embodiment of the method for altering MHC class II antigen presentation of a cell is directed to peptide (antigen) feeding of a MHC class II expressing cell.

Other preferred methods for altering MHC class II antigen presentation of a cell is by altering the cellular level of Ii chain trimers; preferably by altering Ii chain trimer formation; more preferably by administering a therapeutically effective amount of an Ii chain isoform; preferably an Ii chain isoform that contains the ER retention signal, more preferably Ii chain isoform p35 (Ii-p35). Preferably the alteration of cellular Ii chain trimers is achieved by stable transfection of a cell with a nucleic acid that operably expresses an Ii chain isoform (e.g., Ii-p35).

In a related aspect, the invention is directed to a method for treating or slowing the progression of a disease or disorder associated with abnormal MHC class II presentation in a cell by altering MHC class II presentation of the cell. Preferably, the method alters an abnormal MHC class II presentation phenotype (as outlined above) nominally to that of cells from individuals free of the disease or disorder.

Brief Description of the Drawings

Fig. 1. Cell surface binding of biotinylated MHC class II peptides. (A) FACS analyses of binding of biotinylated MHC class II DR-specific peptide HA (307-319) to diabetic twin (solid line) and non-diabetic twin (dotted line) cell lines. (B) Biotinylated MHC class II DR-specific HA peptide binding and IgCκ peptide binding to diabetic (solid) and non-diabetic (hatched) discordant diabetic EBV cell lines. Data are presented as percent increase in fluorescence intensity after peptide addition for a given cell line (background fluorescence subtracted out).

Fig. 2. Total cellular and surface display detection of MHC class II over time. Diabetic twin (DM), non-diabetic twin (T), and control (Con) cells biosynthetically labeled with [S^{35}]methionine and surface-biotinylated at 0, 1, 2 and 3 hours of chase. MHC class II immunoprecipitates are indicative of total MHC class II synthesis (at left), and surface MHC class II (at right).

Fig. 3. MHC class II-peptide display half-life. FACS analyses of disappearance rates of biotin-labeled MHC class II-peptide complexes from the surface of: diabetic twin (◆), non-diabetic twin (■), and control (▲) EBV cell lines over time.

Fig. 4. Pulse-chase results of MHC class II-Ii glycosylation and transport. Arrows indicate the position of the Ii-p35 invariant chain. Bars indicate the positions of the MHC class II α and ß chains after removal of N-linked oligosaccharides with EndoH. Pulse-chase trial results demonstrate MHC class II-Ii chain complexes from diabetic twin (DM) cells rapidly exit the ER, receive decreased carbohydrate addition and acquire rapid EndoH resistance with brief or poor invariant chain association at early time points compared to their matched non-diabetic twins (T).

Fig. 5. MHC class II dimer stability. SDS-PAGE analysis of boiled (B) and non-boiled (NB) MHC class II samples reveal abnormally delayed stable MHC class II dimer formation (αβ) in diabetic twin (DM) cells compared to non-diabetic twin (T) cells.

Fig. 6. Ii-p35 isoform expression levels. Western immunoblot of Ii-p35 and Ii-p41 isoforms, and HCS in cellular lysates from matched diabetic (DM) and non-diabetic (T) twin cells.

Fig. 7. Ii-p35 protein levels in transfected twin cells. Western blot analysis of Ii-p35 and control HC3 protein levels in diabetic twin (DM), non-diabetic twin (T), and non-diabetic control (Con) untransfected with Ii-p35 cDNA, compared to Ii-p35 cDNA transfected non-diabetic twin (Tp35) and diabetic twin (DMp35) cell lines.

Fig. 8. Cell surface binding of biotinylated MHC class II peptides in Ii-p35 transfected cells. FACS analyses of binding of biotinylated MHC class II DR specific peptide HA (307-319) to diabetic twin (solid line) and non-diabetic twin (dotted line) cell lines. (A) Non-Ii-p35 transfected diabetic twin cells (DM) compared to non-Ii-p35 transfected non-diabetic twin cells (T). (B) Non-Ii-p35 transfected diabetic twin cells (DM) compared to Ii-p35 transfected diabetic twin cells (DMp35). (C) Non-Ii-p35 transfected non-diabetic twin cells (T) compared to Ii-p35 transfected diabetic twin cells (DMp35). (D) Ii-p35 transfected non-diabetic twin cells (Tp35) compared to Ii-p35 transfected diabetic twin cells (DMp35). Background fluorescence for all cell lines was less than 4% (data not shown).

Fig. 9. Total cellular and surface display detection of MHC class II over time in Ii-p35 transfected cells. Diabetic twin (DM), non-diabetic twin (T), and Ii-p35 transfected diabetic twin cells (DMp35) cells biosynthetically labeled with [S^{35}]methionine and surface-biotinylated at 0, 1, 2 and 3 hours of chase. MHC class II immunoprecipitates are indicative of total MHC class II synthesis (at left), or surface MHC class II (at right).

Detailed Description of the Invention

T cell recognition of surface displayed MHC class I or II antigen complexes requires proper peptide loading within the cleft of a MHC molecule and transport of the complex to the membrane of the cell. MHC class I and class II molecules associate with peptides that have been processed in different intracellular compartments. MHC class II molecules bind peptides derived from exogenous antigens, which are internalized by phagocytosis or endocytosis and processed within the endocytic pathway. Proper intracellular processing of antigenic peptides for MHC class II complexation and display (MHC class II presentation) requires several steps, disruption of any one of which results in abnormal and ineffective MHC class II presentation of T_H cell recognition. As reported herein for the first time, faulty or abnormal MHC class II presentation is characteristic of (and diagnostic for) diseases and disorders associated with MHC class II presentation, including Type I diabetes.

Under normal circumstances, once an antigen is internalized, it is degraded into peptides within endosomal compartments of the endocytic processing pathway. Internalized antigens progress from early to late endosomes (and finally to lysosomes), are exposed to hydrolytic enzymes and decreasing pH. Internalized antigen is degraded into peptides of about 13-18 residues in preparation for loading into the binding groove of MHC class II molecules.

MHC class II formation and transport to the cell surface starts in the ER. Typically, MHC class II molecules are transported through the Golgi complex to the endocytic pathway,

where MHC class II peptide loading takes place. Properly loaded mature MHC class II-antigen peptide complexes are displayed on the cell surface for T cell education and/or stimulation. Displayed MHC class II molecules also undergo "turnover", wherein surface MHC class II is internalized, typically back to the endo/lysomes of the endocytic pathway (for supplemental peptide loading).

Some MHC class II molecules of the Golgi complex may bypass the endocytic pathway, transporting instead directly to the cell membrane. This MHC class II route results in MHC class II-empty or MHC class II-Ii display. Only upon re-routing to the endocytic pathway can these MHC class II molecules become properly complexed with antigen peptides.

The following illustrative explanations are provided to facilitate understanding of certain terms and phrases frequently used and of particular significance herein.

For the purposes of this disclosure, "MHC class II presentation" is used generically to encompass all components of MHC class II complex formation, processing and display. For the purposes of this disclosure, all aspects and components of MHC class II presentation are classified as part of MHC class II "assembly", "transport", or "display".

"MHC class II assembly" includes all aspects of the MHC class II heterodimer formation as well as the formation of all MHC class II accessory components, including but not limited to: invariant chain (Ii chain) trimer formation, MHC class II-Ii chain complex formation, and MHC class II-peptide complex formation or MHC class II peptide loading. Improper MHC class II assembly can lead to abnormal MHC class II display and, therefore, abnormal T_H cell recognition and/or stimulation. Abnormal MHC class II assembly includes but is not limited to: abnormal MHC class II $\alpha\beta$ heterodimer formation, abnormal Ii heterotrimer formation, abnormal MHC class II- Ii chain nonameric complex formation, abnormal MHC class II-CLIP complexation, and abnormal MHC class II peptide loading (i.e., improper MHC class II antigen complex formation).

"MHC class II transport" refers to intracellular movement of the MHC class II protein, Ii chain peptides, and antigen processing. This includes transport within and exit from the endoplasmic reticulum (ER); routing through the Golgi complex (or bypassing the Golgi altogether); the endocytic processing pathway; and transport to the plasma membrane of a cell. The endocytic pathway refers to transport through endocytic vesicles, which are conventionally characterized (based upon increasing acidity) in terms of three compartments: early endosomes (pH ~6.0-6.5), late endosomes or endolysosomes (pH ~5.0-6.0), and lysosomes (pH ~4.5-5.0). Depending on the internalized peptide, MHC class II transport may take 1-3 hours for proper antigen display (i.e., loaded in a MHC class II protein) at the cell surface. Deviations in the

timing and/or route of intracellular compartmentalization (e.g., MHC class II bypass of the Golgi complex and/or endocytic pathway) result in abnormal MHC class II presentation.

"MHC class II display" specifically refers to localization of the MHC class II protein on the surface of an APC. Normal MHC class II display refers to display of MHC class II properly loaded with an antigenic peptide product of the endocytic processing pathway. MHC class II display phenotypes include but are not limited to: MHC class II-empty display, MHC class II display of, e.g., an Ii chain or fragment thereof (e.g., CLIP), MHC class II surface arrival, MHC class II turnover, and MHC class II half-life.

"Abnormal MHC class II presentation" is understood by practitioners in the art to refer to any qualitative (e.g., type or form of) or quantitative (e.g., amount or rate) deviation in MHC class II presentation compared to normal MHC class II presentation of a typical APC, e.g., APC's of an individual free of autoimmune disease. For the purposes of this disclosure, abnormal MHC class II presentation may be caused by any qualitative or quantitative alteration in the normal cellular processes of MHC class II assembly, transport, or display. For diagnostic purposes of a disease or disorder associated with abnormal MHC class II presentation, abnormal MHC class II presentation is determined as compared to that of individuals not afflicted with the disease or disorder.

Four predominant invariant (Ii) chain isoforms are currently known to exist. The four isoforms are the result of two independent gene expression phenomena: an alternate splice variant, wherein an exon (exon 6b, encoding a 64 amino acid peptide) is retained in the mRNA and translated, and an alternate N-terminal translational start site upstream from the conventional start site, wherein an additional N-terminal 16 amino acid peptide is translated. The alternate translational start peptide contains an ER retention signal peptide (Arunachalam et al., 1994). For the purposes of this disclosure, the four Ii isoforms are designated and characterized based upon the presence (+) or absence (-) of the ER retention signal peptide and exon 6b as in Table 1 below:

TABLE 1: Characterization of Invariant Chain Isoforms

Ii chain isoform	N-terminal ER retention peptide (alternate N-terminal start site)	Exon 6b
Ii-p43	(+)	(+)
Ii-p41	(-)	(+)
Ii-p35	(+)	(-)
Ii-p33	(-)	(-)

Following the convention of practitioners in the art, and for the purposes of this disclosure, "APCs", or antigen presenting cells, refer to cell types that normally display MHC class II molecules on their surface. A variety of cells can function as APCs. The definitive characteristic of an APC is the ability to express MHC class II molecules. "Professional APCs" are defined herein as the group of cells consisting of dendritic cells, macrophages, and B cells. "Nonprofessional APCs" refer to other cell types that can be induced to express MHC class II molecules, but typically do so only for short periods of time during a sustained inflammatory response. Nonprofessional APCs include the group of cells consisting of fibroblasts, glial cells, pancreatic β cells, thymic epithelial cells, thyroid epithelial cells, and vascular endothelial cells.

For the purposes of practicing the present invention, "providing an antigen presenting cell" refers to providing any sample derived from a mammal, which sample contains an APC of the mammal or a cellular lysate or cellular extract, which contains molecular components representative of a MHC class II presentation phenotype of an APC from the mammal.

An "abnormal MHC class II presentation phenotype" refers to any detectable cellular or biochemical characteristic of abnormal MHC class II presentation (as described earlier).

As defined herein, a "disease or disorder associated with abnormal MHC class II presentation" refers to any acute or chronic disease or disorder of an individual that is characterized by abnormal MHC class II presentation. The pathology of such diseases or disorders manifests itself in faulty T cell education and/or inappropriate T cell immune response. Abnormal MHC class II presentation is characteristic of the penetrance of immune-related diseases, such as autoimmune diseases. The discoveries disclosed herein particularly implicate Type I diabetes as one disease associated with abnormal MHC class II presentation.

"Treating a disease or disorder" associated with abnormal MHC class II presentation generally refers to any process that functions to slow, to halt (including stopping initial onset), or to reverse the adverse clinical consequences following from unchecked abnormal MHC class II presentation.

As used herein, a "therapeutically effective amount" is an amount effective to achieve the desired physiological result in a subject. For example, a therapeutically effective amount of Ii-p35 chain is an amount sufficient to alter an otherwise abnormal MHC class II presentation phenotype to that exhibited by a normal APC for a period of time sufficient to ameliorate one or more of the pathological processes associated with abnormal MHC class II presentation. The effective amount will vary depending on the specific therapeutic agent selected and its mode of delivery. An effective amount is also dependent on a variety of factors and conditions related to

the subject to be treated (for example, the age, weight and health of the patient as well as dose response curves and toxicity data) and the severity of the disorder (e.g., a pre-diabetic stage would require lesser treatment, for example, the elimination of a small number of autoreactive cells, than late stage diabetes). Determination of a pharmaceutically effective amount for a given agent is well within the ability of those skilled in the art.

"Administration" to an individual is not limited to any particular delivery system and may include, without limitation, parenteral (including subcutaneous, intravenous, intramedullary, intraarticular, intramuscular, or intraperitoneal injection) rectal, topical, transdermal or oral (for example, in capsules, suspensions or tablets) delivery. Administration to an individual may occur in a single dose or in repeat administrations, and in any of a variety of physiologically acceptable salt forms, and/or with an acceptable pharmaceutical carrier as part of a pharmaceutical composition. Physiologically acceptable salt forms and standard pharmaceutical formulation techniques are well known to persons skilled in the art (see, for example, Remington's Pharmaceutical Sciences, Merck Publishing Co.). Administration of a therapeutic agent to an individual may also be by means of gene therapy, wherein a nucleic acid sequence encoding the agent is administered to the individual *in vivo*, or to cells *in vitro* (which are then introduced into an individual); the agent is produced by expression of the product encoded by the nucleic acid sequence. Methods for gene therapy are also well known to those of skill in the art (See, for example, Border, 1996).

As used herein, an "individual" refers to any mammalian subject that may be afflicted with or potentially susceptible to a disease or disorder associated with abnormal MHC class II presentation.

Described herein is the discovery that particular isoforms of the invariant (Ii) chain are important for proper MHC class II presentation; specifically, those Ii isoforms that contain the ER retention signal peptide located at the N-terminal region of the protein and incorporated by virtue of an alternate, upstream translational start site. Such ER retention signal-containing Ii isoforms include Ii-p35 and Ii-p43. Abnormally reduced cellular levels of these ER retention signal-containing Ii isoforms (utilized as at least one subunit of the naturally assembling Ii trimer molecule) lead to a host of abnormal MHC class II presentation phenotypes, which result in faulty MHC class II display. This faulty display affects the immune (T cell) education and stimulation processes in an individual and eventually manifests itself as a disease state in the individual. Such disease states are characterized by inappropriate or undesirable immune responses and particularly include many autoimmune diseases. Detection of any one or more of these abnormal MHC class II phenotypes, therefore, is indicative of a disease associated with

abnormal MHC class II presentation. Similarly, correction of any one or more of these abnormal MHC class II phenotypes is therapeutic for the disease state.

According to the discoveries described herein, detection of abnormal MHC class II presentation phenotypes provides a means for detecting and measuring penetrance of a disease state associated with abnormal immune response. Disease penetrance refers to the development of disease-related phenotype leading to pathology and signaling onset of disease. Individuals possessing a genetic predisposition for a disease may nonetheless not manifest symptoms of the disease, until penetrance occurs and pathological symptoms of the disease manifest themselves. The present invention provides a means for monitoring disease penetrance of immune-related disorders, such as Type I diabetes, by detecting abnormalities in MHC class II antigen presentation. Preferably, penetrance can be detected in this way before irreversible pathology has occurred, and most preferably such detection can point to therapeutic steps that can be taken to delay onset of the disease or even eliminate the phenotypes accounting for penetrance of the disease.

Diabetes mellitus is a disease that affects at least 2% of the U.S. population. Diabetes mellitus is characterized by hyperglycemia (increased blood glucose). Although initially asymptomatic, diabetes can lead to a variety of complications and secondary disorders including: cataracts; microangiopathy (retinopathy, glomerulosclerosis); arteriopathy (cerebral, coronary, peripheral); hypertension; neuropathy (peripheral, spinal cord or roots, autonomic nervous system, cranial nerves); amyotrophic lateral sclerosis; various genitourinary disorders (Candida vulvovaginitis, Candida balanitis, bacterial bladder or kidney infection, renal failure, nephrotic syndrome); Dupeytren's contracture; xanthomatosis; various skin disorders; and other secondary diseases including periodontal disease, tuberculosis, and mucormycosis. There are four types of diabetes mellitus, two of which account for the vast majority of cases.

Type I (or Type 1) diabetes, also referred to as insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes, is one manifestation of autoimmune disease and accounts for about 5-10% of all diagnosed cases of diabetes. Risk factors are less defined for Type I diabetes than other forms of diabetes, but genetic, autoimmune, and environmental factors are believed to be involved in its onset and progression. The proximate cause of Type I diabetes appears to be an autoimmune destruction of the insulin-secreting β cells (found in the islets of Langerhans) of the pancreas. A genetic correlation has been reported between Type I diabetes and the genes encoding MHC class II polypeptides (Todd et al., 1987; Thomson et al., 1988; and Baisch et al., 1990). Despite the apparent genetic predisposition to the disease, however, identical twin studies reveal only a 40% concordance (Kyvik, 1995; Kumar, 1993; Srikanta, 1983; Redondo, 1999;

Verge, 1995; Petersen, 1997; Hawkes, 1997; Rowe, 1995; Leslie, 1996; Gottlieb, 1968; Pyke, 1976; Tattersall, 1973); that is, less of a correlation than that found for other traits such as handedness, high blood pressure, tuberculosis, or even Type II diabetes.

Current diagnostic tests for diabetes include a fasting plasma glucose test in addition to the previously preferred (but more difficult and costly) oral glucose tolerance test. A confirmed fasting plasma glucose value of greater than or equal to 126 milligrams/deciliter (mg/dL) indicates a diagnosis of diabetes (especially with the presence of ketones). In the presence of symptoms of diabetes, a confirmed nonfasting plasma glucose value of greater than or equal to 200 mg/dL indicates a diagnosis of diabetes. An oral glucose tolerance test (by administering 75 grams of anhydrous glucose dissolved in water, in accordance with World Health Organization standards, and then measuring the plasma glucose concentration 2 hours later) value of greater than or equal to 200 mg/dL indicates a diagnosis of diabetes.

Treatment strategies for diabetes are continually being developed and revised. Current research concerning the progression and treatment of diabetes has focussed on mechanisms to maintain blood glucose near normal levels at all times. Treatment of Type I diabetes, which results from diminished insulin production by the pancreas, is particularly difficult to control; requiring a rigid regimen that typically includes a carefully controlled diet, a strict exercise regime, constant monitoring of blood glucose levels, and obligatory daily insulin injections for life.

Abnormal MHC class II presentation phenotypes that are detected according to the methods described herein involve abnormalities in MHC class II assembly, MHC class II transport, and MHC class II display. Examples of such phenotypes include:

- abnormal (e.g., inadequate or reduced) MHC class II-Ii chain glycosylation;
- abnormal antigen peptide loading (e.g., reduced MHC class II-peptide loaded display, decreased MHC class II-CLIP fragment display, increased MHC class II-empty display);
- abnormal MHC class II heterodimer stability (e.g., delayed SDS-PAGE stability);
- abnormal Ii chain isoform levels (e.g., reduced Ii chain isoform possessing the N-terminal ER retention signal, abnormal cellular level of Ii-p35 containing Ii chain trimer, reduced cellular level of Ii-p35 isoform, reduced cellular level of Ii-p45 isoform);
- abnormal (e.g., increased) rate of MHC class II complex exit from the ER or other intracellular compartment;
- abnormal route of MHC class II complex compartment localization (e.g., bypassing the Golgi complex);
- abnormal (e.g., increased) rate of MHC class II complex endosomal degradation;
- abnormal (e.g., increased) rate of MHC class II complex surface arrival;
- abnormal (e.g., increased) rate of MHC class II complex surface turnover; and
- abnormal (e.g., decreased) MHC class II half-life.

MHC class II presentation errors indicative of diseases and disorders expressive in humans were investigated using immortalized Epstein Bar virus (EBV) B cell lines derived from identical twins discordant for Type I diabetes. Paired identical twin studies using EBV B cell lines with discordant disease expression eliminate confounding genetic influences of the disease produced by the complex and diverse MHC class II haplotypes (conferred by unique combinations of MHC class II α and ß gene products). By studying identical twin cells, the (epigenetic) influence of abnormal MHC class II assembly, transport, or display specific to disease expression, is examined independently of MHC class II allelic factors.

Examples, disclosed herein, were performed on human B cells as the preferred antigen presenting cell (B cells internalize antigen efficiently by receptor-mediated endocytosis using antigen-specific membrane antibody as the receptor to capture the antigen). It is, however, well within the skill in the art to practice the present invention using any APC type, and derived from any animal species possessing APCs (as desired). Notably, for human B cells, the dominant invariant chain isoforms are Ii-p33 followed by Ii-p35 forms, with lesser amounts of the Ii-p43 and Ii-p45 isoforms. Examples, therefore, focus on the Ii-p33 and Ii-p35 isoforms as illustrative, not limiting, of the present invention.

Since APCs express both class I and class II MHC molecules, and because MHC class II formation occurs in the ER together with MHC class I molecules and endogenous antigens normally destined for MHC class I presentation, a cellular mechanism exists to prevent MHC class II molecules from binding to the same set of antigenic peptides as that of MHC class I molecules. The invariant chain (Ii) is an important chaperone protein in both the intracellular transport of MHC class II proteins and in the regulation of peptide loading to ensure a diverse repertoire of peptides for presentation to $CD4^+$ T cells (Cresswell, 1994). Within the ER, the Ii chain typically forms a core trimer, which in turn associates with three MHC class II αß dimers, resulting in a nonameric complex $(\alpha\beta I)_3$ (Roche, 1991; Lamb, 1992). This complex is incapable of binding antigenic peptides, an important feature that prevents endogenous peptides (typically destined for MHC class I loading) from loading the MHC class II groove while in the ER (Roche, 1990; Newcomb, 1993).

In human lymphocytes and immortalized B cells, two alternative Ii isoforms function in the transport and intracellular routing of MHC class II from the ER to the endosomal peptide loading compartment. The predominant Ii chain is Ii-p33, but Ii-p35, generated by the use of an alternative upstream translation initiation site, is also expressed in lesser quantities (Strubin, 1986). The two Ii isoforms are identical in all coding regions except that Ii-p35 contains an additional 16 amino acid peptide at the cytoplasmic N-terminus.

The ratio of Ii isoforms in fixed lineage cells is tightly regulated. Ii-p35 represents 20% of the total cellular amount Ii in normal human B cells. The majority of total MHC class II-Ii complexes contains at least one Ii-p35 polypeptide as a component of the Ii trimer (Anderson, 1999; Roche, 1991; Lamb, 1992). The present invention relates, in part, to the discovery that the Ii-p35-containing trimer (and hence a minimal threshold amount of cellular Ii-p35) is important for normal MHC class II antigen presentation.

The chaperone function of Ii-p35 versus Ii-p33 in MHC class II transport and assembly is fundamental to intracellular antigen processing events including the intracellular processing pathway used by MHC class II molecules for peptide loading and routing of MHC class II molecules to the cell surface for antigen display. The ratio of the two invariant isoforms also affects the choice and timing of compartment localization, the rate of ER exit, the amount of post-Golgi glycosylation, the rate of intracellular peptide loading, and the degree of endosomal degradation. MHC class II complexed with a mixed Ii trimer containing Ii-p35 are routed to the endosome/lysosome directly from the Golgi complex, whereas MHC class II complexed to Ii-p33 reach the endosomal/lysomal system via the plasma membrane (i.e., prior to peptide loading in the endosome/lysosome). (See, Lamb, 1991; Leslie & Pyke, 1996; Roche, 1993; Saudrais, 1998; Tattersall & Pyke, 1973; Wang, 1997; and Warmerdam, 1996.) The range of MHC class II transport kinetics, from slowest to fastest, is shown by cells exclusively expressing Ii-p35, exclusively expressing Ii-p33, or lacking any Ii chain (Arunachalam, 1994). Normally, MHC class II is retained in the ER through the formation of mixed Ii trimers containing at least one Ii subunit having the ER retention signal (N-terminal 16-mer peptide). Normal or excess expression of Ii-p35 chain proteins in transfected cells ensures normal MHC class II presentation (Romagnoli, 1993).

The Ii chain contains signal peptides in its cytoplasmic tail, which directs the transport of MHC class II from the trans-Golgi network to the vesicular compartments of the endocytic pathway. En route to the endosomes of the endocytic pathway, the MHC class II-Ii complex undergoes extensive glycosylation and phosphorylation (Bakke, 1990; Lotteau, 1990; and Lamb, 1991). MHC class II-Ii chain glycosylation provides a protective benefit upon arrival to the endosome. Proteins possessing complex glycosylation, in the endosome, block or slow the activity of asparagine specific cysteine endopeptidase, AEP, an important lysosomal/endosomal enzyme that facilitates complete MHC class II peptide presentation (Manoury, 1998).

Glycosylation and phosphorylation patterns are altered by the absence of MHC class II-Ii complexes, wherein the Ii trimer contains at least one Ii-p35 isoform (Kuwana, 1998; and Anderson, 1999). Diabetic cells predominantly express Ii-p33, a cellular phenotype that hastens

ER exit and eliminates complex glycosylation of the asparagine residues at position 114 and 120 of the invariant chain (Claesson et al., 1983; Peterson, 1987; Peterson, 1990; Peterson, 1992; Pyke et al., 1976; Tattersall & Pyke, 1972; and Tattersall & Pyke, 1973). This simple glycosylation pattern is commonly measured by resistance to EndoH digestion, which is indicative of rapid intracellular transport. The presence of at least one Ii-p35 invariant isoform with ER retention signals slows ER exit of the MHC class II assembly, which facilitates complex glycosylation (exemplified by sensitivity to EndoH digestion) as a result of delayed ER exit (Lotteau, 1990).

Within the endosomal compartments, Ii is partially degraded, leaving MHC class II molecules bound to an Ii fragment, called CLIP (for CLass II-associated Invariant chain Peptide). CLIP physically occupies the peptide-binding groove of the MHC class II molecule, and continues to prevent MHC class II peptide loading. A MHC class II-like molecule (HLA-DM) functions in the removal of CLIP and permits the subsequent antigen peptide loading of MHC class II. HLA-DM is also an αβ heterodimer, however it is not displayed at the cell membrane but is instead found predominantly within endosomal compartments (see, Denzin, 1995; Sherman, 1995; and Sloan, 1995). CLIP removal results in the association of high affinity peptides with MHC class II, which confers MHC class II stability to the peptide-containing complex (commonly measured by resistance to dissociation with boiling in SDS-PAGE gels).

The rate of CLIP removal may additionally be influenced by a given MHC class II allele and the binding affinity of that MHC class II molecule to CLIP (Sette, 1995; Buckner, 1996). In addition, the rate of cleavage and peptide loading in the endosomal/lysosomal compartment is regulated by a variety of proteases; in particular, asparaginyl endopeptidase (AEP), a cysteine protease involved in MHC class II glycosylation (AEP activity is decreased by the high degree of ER glycosylation of the delivered protein substrates; Manoury, 1998).

Description of the Preferred Embodiments

Based upon the discoveries reported here for the first time, effective detection of an abnormal MHC class II presentation phenotype is indicative of an individual afflicted with or predisposed to develop a disease associated with abnormal MHC class II presentation. Furthermore, correction (i.e., alteration to within normal parameters) of any one or more of these abnormal MHC class II presentation phenotypes can serve as treatment for such a disease, e.g., Type I diabetes associated with faulty MHC class II presentation.

DETECTION METHODS

The present invention provides an assay for determining the predisposition to develop a disease or disorder associated with abnormal MHC class II presentation by detecting an abnormal MHC class II presentation phenotype.

MHC class II presentation phenotypes useful as a marker for diseases or disorders associated with abnormal MHC class II presentation may be based upon any detectable or measurable aberration in MHC class II assembly, transport, or display. Such measurable MHC class II presentation phenotypes include but are not limited to abnormal MHC class II-Ii chain glycosylation; abnormal antigen peptide loading (e.g., reduced MHC class II-peptide loaded display, decreased MHC class II-CLIP fragment display, increased MHC class II-empty display); abnormal MHC class II heterodimer stability (e.g., delayed SDS-PAGE stability); abnormal Ii chain isoform levels (e.g., reduced Ii chain isoform possessing the N-terminal ER retention signal, abnormal cellular level of Ii-p35 containing Ii chain trimer, reduced cellular level of Ii-p35 isoform, reduced cellular level of Ii-p45 isoform); abnormal rate of MHC class II complex exit from the ER or other intracellular compartment; abnormal rate or route of MHC class II complex compartment localization (e.g., exiting the Golgi too soon or bypassing the Golgi complex); abnormal rate of MHC class II complex endosomal degradation; abnormal rate of MHC class II complex surface arrival; abnormal rate of MHC class II complex surface turnover; and abnormal MHC class II half-life.

Detection includes any method known in the art useful to indicate the presence, absence, or amount of a detection target. Such methods may include, but are not limited to, any molecular or cellular techniques, used singularly or in combination, including, but not limited to: hybridization and/or binding techniques, including blotting techniques and immunoassays; labeling techniques (chemiluminescent, colorimetric, fluorescent, radioisotopic); spectroscopic techniques; separations technology, including precipitations, electrophoresis, chromatography, centrifugation, ultrafiltration, cell sorting; and enzymatic manipulations (e.g., digestion). Especially preferred techniques and reagents are described below.

Antibody probes

Antibodies are useful for measuring many MHC class II presentation phenomena. Polyclonal or monoclonal antibodies (MAbs) may be used. Antibodies useful for MHC class II phenotype detection may be generated using immunization and hybridoma techniques well known in the art or may be purchased from commercial venders (e.g., Coulter, Hialeah, FL).

For the preparation of monoclonal antibodies, any technique that provides antibodies produced by continuous cell line cultures can be used. Well known examples of such techniques

include the hybridoma technique (Kohler and Milstein, 1975), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985). Techniques for generating antibody fragments and single-chain antibodies (as described in U.S. 4,946,778) can be adapted to produce suitable probes recognizing immunogenic peptide components of MHC class II presentation, and as such will be useful for identifying MHC class II presentation phenotypes described herein.

Many MAbs useful in the practice of the invention have been reported and are available from various sources. Preferred MAbs include but are not limited to: **DA6.147** (Guy, 1982); **16.23** (a gift of Dr. Rudolf Wank); **L243**, which predominantly recognizes αß dimers of MHC class II (DR) devoid of intact invariant chain (ATCC accession # HB-55, Manassas, VA); **I 9.3F10**, which recognizes all HLA class II gene (DR, DP, and DQ) products (Van Voorhis, 1983b); **I-2**, which recognizes HLA-DR; mAb16.23, which recognizes the MHC class II DR3 dimer (Mellins, 1990; Riberdy, 1992); **I-3**, which recognizes a nonpolymorphic region of MHC class II allowing binding to all class II genes (DR, DP and DQ); **PIN.1**, which detects the cytoplasmic N-terminal portion of Ii-p33 and Ii-p35 (a gift of Dr. Peter Cresswell, Yale University); and **CerCLIP.1**, which recognizes the CLIP fragment of the invariant chain in association with MHC class II (Peter Cresswell). Preferably, immunodetection utilizes cell sorting, or blotting techniques well known in the art.

Peptide feeding

Another approach useful to detect abnormal MHC class II presentation is to comparatively quantify peptide occupancy of surface MHC class II structures. A preferred technique is to quantify the relative fraction of surface MHC class II proteins that can be filled with exogenously labeled MHC class II binding peptides. This technique utilizes the ability of MHC class II-empty surface complexes to bind exogenous peptide added to tissue culture media (see Peterson, 1990; Bikoff et al., 1993; Viville, 1993; Elliott, 1994). All three MHC class II αß dimers (DR, DP and DQ), improperly assembled en route to the cell surface, easily fill with exogenously supplied peptide exposed to surface expressed MHC class II proteins. Labeled peptide feeding of cell cultures can be used to quantify (previously empty) MHC class II-labeled peptide complexes. A preferred detection technique utilizes flourescently activated cell sorting (FACS) protocols well known in the art.

Pulse-chase techniques

MHC class II phenotypes can be measured based upon localization of events in time or space or as a rate of change over time. Pulse-chase techniques well known in the art may be employed to measure various cellular events by exposing living cells to a "pulse" of labeled

substrate for a fixed period of time, followed by a saturating amount of unlabeled substrate. Such techniques are useful to measure, e.g., MHC class II surface arrival, turnover, and half-life.

Enzymatic detection

MHC class II presentation states can also be measured by sensitivity or resistance to enzymatic action (e.g., digestion). For example, EndoH (as discussed earlier) is an enzyme that cleaves early carbohydrate additions to MHC class II-Ii complexes. Sensitivity to EndoH digestion (i.e., cleavage of MHC class II-Ii glycosylation resulting in lowered MHC class II-Ii complex molecular weight), or conversely EndoH resistance, is a known effective technique to trace the rate and route of intracellular MHC class II transport.

SDS-PAGE analysis

As mentioned earlier, boiling SDS polyacrylimide gel electrophoresis (SDS-PAGE) treatment is a method well known to practitioners in the art as useful to measure by MHC class II dimer resistance to dissociation (i.e., MHC class II dimer stability; see, e.g., Kumar et al., 1993).

Western Blot techniques

Finally, in combination with several of the above-mentioned detection reagents and separation techniques, western blotting techniques well known in the art are highly valuable preferred techniques in the qualitative and quantitative analyses of cellular proteins.

TREATMENT METHODS

In view of the discoveries reported herein for the first time, in addition to effective detection of an abnormal MHC class II presentation phenotype as indicative of an individual afflicted with or predisposed to develop a disease or disorder associated with abnormal MHC class II presentation, correction (i.e., alteration to within normal parameters) of any one or more of these abnormal MHC class II phenotypes can serve as a treatment for a disease or disorder associated with faulty MHC class II presentation, such as Type I diabetes.

The present invention provides a method for altering MHC class II presentation by altering one or more MHC class II presentation phenotypes. In a preferred embodiment, alteration of a MHC class II presentation phenotype effectively restores an otherwise abnormal MHC class II display of a cell to normal, thereby being therapeutic for a disease or disorder associated with faulty MHC class II display.

A person skilled in the art will appreciate that effective alteration of any one of the MHC class II phenotypes identified above will ameliorate the deleterious effects of abnormal MHC class II display and will reverse the APC-mediated immune response (or unresponsiveness) that results from such abnormal display. Specific alteration of MHC class II presentation includes but

is not limited to: increasing MHC class II-Ii glycosylation, increasing MHC class II dimer stability, decreasing MHC class II endosomal degradation, increasing cellular levels of an N-terminal ER retention signal-containing Ii isoform (e.g., Ii-p35 and/or Ii-p43), decreasing the rate of MHC class II transport from the ER, decreasing the amount of MHC class II-empty surface display, decreasing the rate of MHC class II arrival, decreasing the rate of MHC class II turnover, and increasing MHC class II half-life.

Any of a variety of techniques known in the art may be used to achieve the above alteration of abnormal MHC class II presentation phenotypes to normal ranges. Preferred techniques are disclosed and exemplified herein, but these are not limiting for the present invention.

Ii chain supplementation

A fundamental error resulting in a disease or disorder associated with abnormal MHC class II presentation (as identified herein) is a disproportionate expression of an alternate translational Ii chain mRNA transcript, which effectively converts an at-risk disease genotype into actual disease expression. The N-terminal ER retention signal-containing Ii isoform is a significant component of the Ii chain trimer; and the absence of the ER retention signal results in abnormal MHC class II presentation, followed by a breakdown in T_H cell education and/or stimulation, resulting in a disease state. Treatment of a disease or disorder associated with MHC class II presentation includes a method for altering MHC class II display by supplementing a cell with an amount of an ER retention signal-containing Ii isoform (e.g., Ii-p35 or Ii-p45, or both) sufficient to alter the MHC class II display by the cell.

Ii supplementation may be achieved by providing to a cell effective amounts of synthetically produced or isolated peptide; e.g., Ii chain trimer molecules, wherein the trimer contains at least one Ii isoform having an ER retention signal peptide. Alternatively, supplementation with an ER retention signal containing Ii isoform is possible. The polypeptides may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, fungal, higher plant, insect, or mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated.

Ideally, the treatment method of the present invention would provide long lasting effect. Therefore, the most preferred treatment method for Ii chain supplementation would include regulating the expression of the various Ii isoforms ("regulating expression" is understood by persons skilled in the art to refer to any process to control or modulate the quantity or activity

(functionality) of a peptide in a cell). Such regulating mechanisms are well known in the art and include but are not limited to: altering endogenous Ii gene expression, and/or providing an exogenous nucleic acid which encodes an appropriate Ii isoform, in an expressible form (e.g., in an expression vector), and/or altering the translation machinery in the cell to complex with and translate the ER retention signal-containing Ii chain isoform.

Polynucleotides encoding an Ii isoform of interest are utilized in accordance with the present invention for causing expression of the Ii isoform *in vivo*. For example, cells may be engineered *in vivo* for expression of an Ii isoform *in vivo* by procedures known in the art. For instance, a cell producing a retroviral particle containing RNA encoding the Ii isoform of interest may be administered to an individual at risk for or afflicted with a disease or disorder associated with abnormal MHC class II display for infection and transformation of the individual's cells *in vivo* and expression of the particular Ii isoform *in vivo*. Alternatively, known microinjection techniques can be employed to insert plasmid DNA into cells of a patient, thereby augmenting Ii chain expression of those cells.

Cells from an individual at-risk for or afflicted with a disease or disorder associated with abnormal MHC class II display may also be transfected with a polynucleotide (DNA or RNA) encoding a Ii isoform *ex vivo* (using any one of a variety of techniques well known in the art). The resultant transfectants expressing the introduced Ii isoform polynucleotide can reverse a Ii isoform deficiency in the host cell, or supplement low expression of a particular isoform by the host cell, or provide an additional Ii isoform in the cell, thus augmenting Ii chain cellular levels naturally expressed by the host cell. The transfectants then can be reintroduced into the individual for disease treatment.

For example, one treatment method would include transfection of an APC with cDNA expressing Ii-p35 into affected cells to restore MHC class II display to normal. Such a treatment method by the stable transfection with an Ii-p35 construct is demonstrated herein. Stable transfection of diseased cells of a subject diagnosed with Type I diabetes restored MHC class II assembly, transport and display phenotypes to those comparable to cells of a non-diseased (non-diabetic) identical twin.

These and other methods for administering polynucleotides or using a polypeptide of the present invention will be apparent to those skilled in the art from the foregoing description and the examples to follow.

Protease inhibitors

Additional treatment methods involve providing to a cell an effective amount of an inhibitor directed to one or more of the various endosomal proteases involved in MHC class II

presentation. Effective protease inhibitors would slow degradation of poorly glycosylated MHC class II and Ii complexes, and allow peptide-empty and poorly glycosylated MHC class II molecules adequate opportunity to bind peptides in the endosome/lysosome. Persons skilled in the art will appreciate that this approach is separate and distinct from other proposed protease inhibitor designs for autoimmune diseases that seek to degrade autoantigens, and that do not function to alter (e.g., restore to normal) MHC class II presentation itself.

The reported dominant enzyme that controls the kinetics of MHC class II presentation in the endosome and that is responsible for generating suitable peptides to form complexes with MHC class II molecules and degradation of lysosomally located peptides is asparagenyl endopeptidase (AEP; see, Manoury, 1998). This enzyme activity is inhibited or slowed by the N-glycosylation of asparagine residues on substrate proteins. Failed complex glycosylation of MHC class II-Ii (see the discussion of MHC class II glycosylation patterns above) results in accentuated MHC class II endosomal proteolysis by AEP in the endosome. Inhibition of AEP would slow MHC class II-Ii degradation and allow for proper peptide loading in the endosome.

Although AEP is a preferred target for protease inhibition, other endosomal protease targets include, but are not limited to: other cysteine proteases such as cathepsin S, cathepsin L, cathepsin B, and cathepsin H, cathepsin F, cathepsin Z (*a.k.a.*, cathepsin P and X), cathepsin V (*a.k.a.*, cathepsin L2 and U), cathepsin K (*a.k.a.*, cathepsin O2), cathepsin W (*a.k.a.*, lymphopain), and cathepsin C (*a.k.a.*, dipeptidyl peptidase I); and aspartyl proteases such as cathepsin D and cathepsin E. Further enzymes are likely to be isolated, and it is likely that different cells have alternative forms of these enzymes that degrade Ii to varying degrees.

Many pharmaceutical reagents have been isolated that modify the intracellular course of MHC class II complexes as well as modify the intracellular degradation of MHC class II complexes. Therapeutic inactivation of cysteine proteases results in incomplete degradation of the invariant chain (Villandangos, 1997). For example, the inhibition of Ii processing by the cysteine/serine protease inhibitor leupeptin slows transport of MHC class II to the surface and causes a dramatic and selective accumulation of class II molecules in lysosomes (Brachet, 1997). Leupeptin is a specific inhibitor of the critical proteolytic step required for invariant chain dissociation from the class II antigen dimer thus facilitating antigen presentation (Blum, 1988). This drug alters both the rate and/or efficiency of Ii chain processing, and thus alters the postendosomal sorting of class II molecules.

Lysosomal function can be altered by chloroquine that affects MHC class II antigen transport by slowing the rate of Ii chain dissociation from class II $\alpha\beta$ dimers prior to cell surface display.

Ii chain phosphorylation

Yet another treatment method of the present invention is directed to augmenting Ii chain phosphorylation, effectively increasing peptide loading efficiency of MHC class II. Of the two major Ii isoforms, only Ii-p35 is uniquely phosphorylated by a member of the protein kinase C (PKC) family of serine/threonine kinases (Anderson, 1998). Phosphorylation of Ii-p35 functions to regulate MHC class II transport and peptide loading in the endocytic pathway, and augmentation of Ii-p35 phosphorylation (e.g., by stimulation of PKC) significantly enhances MHC class II peptide loading (Anderson, 1999). Sufficient enhancement of MHC class II peptide loading by Ii phosphorylation augmentation (e.g., using agents that increase phosphorylation, such as PMA (Life Technologies, Gaithersburg, MD), or agents that stimulate PKC activity), as a therapeutic approach, could reduce or mask entirely phenotypic effects of abnormal MHC class II presentation responsible for abnormal MHC class II display.

MHC class II-CLIP binding affinity

Certain MHC class II alleles are statistically correlated with incidence of Type I diabetes. In addition, published reports (unrelated to studies of human MHC class II alleles) have shown dramatic influences of MHC class II allelic polymorphisms on CLIP affinity (Sette, 1995). This suggests that at-risk MHC class II genotypes exhibit hastened CLIP removal from MHC class II molecules in the endosome, providing an exacerbating genetic component to the epigenetic influence of alternate translation errors of the Ii isoform disclosed herein that result in preferential expression of the Ii-p33 isoform. Supplementing a cell with a MHC class II polypeptide that exhibits increased affinity for CLIP compared to that normally found in the cell, would act to slow abnormally rapid CLIP removal from MHC class II and allow for proper MHC class II peptide loading.

Peptide feeding

In addition to other methods for treating a disease or disorder associated with abnormal MHC class II display, "peptide feeding" may be employed to improve or to overcome an otherwise faulty MHC class II peptide display due to abnormally abundant MHC class II-empty surface display. Exogenous peptide binding to MHC class II-empty surface display is well documented (e.g., Busch, 1990a & 1990b; Mozes, 1989). Peptides, which would normally be displayed on the surface of a normal APC for T cell education and/or stimulation, may be provided to the cells for binding to MHC class II-empty surface complexes.

The present invention incorporates by reference in their entirety techniques well known in the field of molecular biology. These techniques include, but are not limited to, techniques described in the following publications:

Ausubel, F.M. et al. eds., Short Protocols In Molecular Biology (4th Ed. 1999) John Wiley & Sons, NY. (ISBN 0-471-32938-X).

Old, R.W. & S.B. Primrose, Principles of Gene Manipulation: An Introduction To Genetic Engineering (3d Ed. 1985) Blackwell Scientific Publications, Boston. Studies in Microbiology; V.2:409 pp. (ISBN 0-632-01318-4).

Miller, J.H. & M.P. Calos eds., Gene Transfer Vectors For Mammalian Cells (1987) Cold Spring Harbor Laboratory Press, NY. 169 pp. (ISBN 0-87969-198-0).

Mayer, R.J. & J.H. Walker eds., Immunochemical Methods In Cell and Molecular Biology (1987) Academic Press, London. 325 pp. (ISBN 0-12480-855-7).

Sambrook, J. et al. eds., Molecular Cloning: A Laboratory Manual (2d Ed. 1989) Cold Spring Harbor Laboratory Press, NY. Vols. 1-3. (ISBN 0-87969-309-6).

Winnacker, E.L. From Genes To Clones: Introduction To Gene Technology (1987) VCH Publishers, NY (translated by Horst Ibelgaufts). 634 pp. (ISBN 0-89573-614-4).

It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the invention described herein are obvious and may be made without departing from the scope of the invention or the embodiments disclosed herein. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting of the invention.

EXAMPLE 1.0: Discordant Diabetic Twin and Control Cells

It currently believed that MHC class II processing and intracellular associations can vary with the MHC class II allelic combinations of the cell (Germain & Hendrix, 1991; Bikoff et al., 1995; Gorga, 1987; and Sette, 1995). The human MHC class II gene contains up to 14 different class II loci clustered into three major subregions; HLA-DR, HLA-DQ and HLA-DP. Each of these subregions contains at least one functional ß locus and one α locus. Because of this complex genetic component, MHC class II presentation was analyzed using genetically matched Epstein-Bar virus (EBV)-immortalized cell lines derived from discordant diabetic identical twin pairs, as well as compared to random control cell lines from non-diabetic subjects. The utilization of cell lines derived from discordant diabetic twins avoids possible variation in MHC class II presentation due to allelic influences, exclusively detecting MHC class II presentation epigenetic phenotypes.

EBV transformed B cell lines from four sets of identical twins (>15 years of age) discordant for Type I diabetes, and random control cell lines, were prepared using techniques well known in the art. Unless reported otherwise, all transformed cells were grown in RPMI 1640 media supplemented with 10% fetal calf serum and 1% Pen/Strep (Mediatech, Herndon, VA).

Non-diabetic twins and controls were autoantibody negative, had normal insulin secretion, and normal fasting glucose levels exceeding 10 years of yearly observation. MHC class II genotypes of the discordant diabetic twins were determined (twin set #1 KPE/KST (DR 4/5); twin set #2 VIM/ROM (DR 3/5); twin set #3 GAW/MAE (DR 3/3); and twin set #4 CHA/CL (DR 4/4)).

For all of the examples disclosed herein, discordant diabetic identical twin cells were paired to eliminate allelic influences of a varying MHC class II binding groove. Standard T-test analyses of data from diabetic twins, non-diabetic twins, and controls were performed when appropriate. Statistical results provided herein report the mean ± one standard deviation.

EXAMPLE 2.0: MHC Class II Display on B Cells

To characterize abnormal MHC class II phenotypes associated with Type I diabetes, a variety of MHC class II display phenotypes were examined in EBV transformed B cell lines (as described in Example 1 above). Among the MHC class II display phenotypes examined were: MHC class II total surface density, MHC class II-CLIP display; MHC class II empty display; and MHC class II surface arrival, turnover rate, and half-life.

EXAMPLE 2.1: MHC Class II Total Surface Density

The capability of APCs to educate and to stimulate (positively or negatively) T cell activity is, in part, proportional to the total number of MHC class II-peptide complexes displayed on the surface of the cells. Therefore, total surface density of MHC class II molecules from diabetics was compared to that of non-diabetics.

A panel of MAbs to MHC class II proteins was used to compare surface density of MHC class II of diabetic cell lines to non-diabetic twin cell lines, and to control cell lines under identical culture conditions. All cell lines were EBV-immortalized. MAbs I-2, 9.3 F10, and I-3 (described earlier), obtained commercially from Coulter Corporation (Hialeah, FL) were used.

To quantify surface density of MHC class II, including Ii fragments (see Example 2.2), an Epics Elite flow cytometer analysis (Coulter Corporation, Miami, FL) was used. 1×10^6 cells were incubated for 30 minutes at room temperature with 1.5-2.0 μg/ml of primary antibody. For

primary antibodies lacking fluorescence, indirect immunofluorescence was utilized. Goat anti-mouse immunoglobulin G (IgG) fluorescein isothiocyanate was used as the secondary antibody in such cases (Coulter Corporation, Miami, FL). Cells prepared for immunoassay were harvested at a stage of log phase with >95% viability. For flow cytometer analysis, >5000 events per sample were quantified. Background immunofluorescence in all cases was <5%. The data are recorded as mean channel fluorescence (MCF); an indicator of epitope density .

The results are presented in Table 2. No statistical difference was observed for overall levels of MHC class II proteins on the cell surface for any of the comparisons.

In contrast, MAb 9.3F10, an antibody with MHC class II, DR and DQ gene product recognition, consistently demonstrated increased surface expression on diabetic cell lines compared to matched non-diabetic twin or control cell lines. Although the exact conformation of MHC class II recognized by MAb 9.3F10 is not known, the antibody recognizes a subset of APCs with enhanced ability to present exogenously added antigen, and provides an indirect indication of preferential peptide MHC class II-empty recognition (Van Voorhis, 1983a).

These flow cytometric results indicate overall MHC class II surface density is normal (not significantly different from non-diabetic twin and control cell line values) in diabetic cell lines, but indicate that peptide MHC class II-empty display on diabetic cell lines is abnormally abundant.

EXAMPLE 2.2: MHC Class II-CLIP Display

MHC class II presentation involves complex intracellular assembly and processing including associations with the chaperone proteins such as the invariant chain. MHC class II processing errors involving chaperones often result in abnormal MHC class II display. In the case of MHC class II-Ii complex aberrations, the CLIP fragment of the Ii chain may remain attached to the MHC class II molecule due to abnormal endosomal/lysosomal cleavage (Sloan, 1995; Denzin, 1995; Fling, 1994; Stebbins, 1996) resulting in abnormally high MHC class II-CLIP surface display. In contrast, abnormally low MHC class II-CLIP surface display is a described trait of human lymphoid cells that are developmentally immature, or have augmented endosomal/lysosomal processing (Harris, 1996 #2338; Santambrogio, 1999a &1999b). To demonstrate that abnormal MHC class II-CLIP display on the surface of an APC is correlated with Type I diabetes, surface display of MHC class II-CLIP was investigated using antibodies to the CLIP fragment.

Monoclonal antibody CerCLIP.1 (provided by Dr. Peter Cresswell, Yale University) recognizes the CLIP fragment of the invariant chain in association with MHC class II and was

used for quantifying the density of the Ii chain complexed to MHC class II and displayed on the surface of an APC. Immunoassay and FACS scan analysis procedures were identical those described in Example 2.1

The results, also presented in Table 2, revealed that all eight diabetic cell lines exhibited abnormally low MHC class II-CLIP display compared to their matched non-diabetic twin cell lines as well as to control cell lines. Data from three repeat analyses confirmed all diabetic cell lines compared to matched non-diabetic twins or control cell lines consistently showed significant reductions in CLIP occupancy of surface MHC class II structures.

TABLE 2. Mean Channel Fluorescence (MCF) of MHC Class II and Invariant Chain Complexes (CLIP) on the Surface of IDDM (Diabetic) Twin, Non-diabetic Twin, and Control Cell Lines.

MAb	IDDM Twin	Non-diabetic Twin	P	IDDM Twin	Non-diabetic Control	P
MHC class II						
L243	17.00	17.02	0.915	17.00	17.05	1.000
(n=8)	(± 0.32)	(± 0.18)		(± 0.32)	(± 0.05)	
I-2	15.71	16.09	0.079	15.71	16.25	0.967
(n=7)	(± 0.86)	(± 1.09)		(± 0.86)	(± 0.35)	
9.3F10	25.2	20.3	0.001	25.2	21.89	0.009
(n=7)	(±0.68)	(±0.825)		(±0.68)	(±0.18)	
I-3	12.30	13.40	0.470	12.3	12.50	0.100
(n=7)	(± 1.30)	(± 0.66)		(± 1.30)	(± 0.01)	
Invariant Chain						
CerCLIP.1	6.80	8.80	0.062	6.80	7.10	0.060
(n=8)	(± 2.20)	(± 3.20)		(± 2.20)	(± 1.5)	

EXAMPLE 2.3: Relative Abundance of MHC Class II-Empty Surface Display

To demonstrate further that abnormal levels of peptide MHC class II-empty display on the surface of an APC is indicative of Type I diabetes, surface display of MHC class II-empty display was quantified for diabetic twin cells, non-diabetic twins cells and control cells.

Diabetic twin cells, non-diabetic twin cells and control cells were incubated in saturating amounts of two different MHC class II antigens. Human HA(307-319) peptide [PKYVKQNTLKLAT; SEQ ID NO:1], derived from influenza A/Texas/1/77 virus H3 hemagglutinin, and human IgC*k*(37-51) peptide [KVQWKVDNALQSGNS; SEQ ID NO:2] are both MHC class II DR restricted peptides. These DR-specific peptides are known to complex with diverse MHC class II DR allelic molecules, but do not complex with MHC class II DQ

binding sites (Busch, 1990b). Peptides were biotinylated at the N-terminus (Quality Control Biochemicals, Inc., Hopkinton, MA) following manufacturer's instructions, and were HPLC purified, lyophilized, and reconstituted in water following standard protein purification procedures known in the art. Paired studies consisted of matched diabetic and non-diabetic twin cells saturation-incubated with each of the two MHC class II specific peptides (independently) for 4 hours.

Assays used to study exogenous peptide binding to MHC class II molecules have been extensively documented in the literature (e.g., Busch, 1990a & 1990b; Mozes, 1989). EBV cells (3×10^5 cells/ml) in 50 µl of complete tissue culture media were combined with 50 µM of biotinylated peptide and incubated at 37°C for 4 hr. After a brief wash, the cells were incubated with fluoresceinated streptavidin (4.22 ug/ml) (Gibco-BRL, Gaithersberg, MD) at 4°C for 30 minutes (as described by Busch, 1990a).

An alternative protocol to 4°C incubation was to treat the cells with sodium azide (12 mM) to prevent endocytosis, and then to incubate with peptide in a 2-hr binding assay. The 4°C incubation and sodium azide treatment demonstrated similar results by flow cytometry.

Stained cells were analyzed on a Coulter flow cytometer (Coulter Corporation, Hialeah, FL). For enhanced peptide detection of MHC class II bound complexes, cells incubated with biotinylated peptides were sequentially incubated at 4°C with fluoresceinated avidin D, with biotinylated anti-avidin D, and then again fluoresceinated avidin D (Vector Labs, Burlington, CA) (see Busch, 1990a).

Overall fluorescence of bound MHC class II-peptide was assessed by flow cytometry for the test antigen. Following standard procedures known in the art for flow analysis, the relative amount of fluoresceinated avidin bound for a set number of cells (5,000 cells/sample) was determined for viable cells and background fluorescence simultaneously quantified and subtracted. Background fluorescence in the absence of biotinylated peptide was determined by incubation with streptavidin alone.

The data are reported in Figure 1 as log fluorescence intensity (Fig. 1A) and percent increase in fluorescence intensity after peptide incubation, and corrected for background fluorescence (Fig. 1B). The Data represent duplicate trials performed on the same day. Trials were repeated three times with similar results (data not shown).

MHC class II-HA(307-319) binding was significantly greater for diabetic twin cells compared to non-diabetic twin cells in all of the discordant twin sets tested; indicating peptide filling of MHC class II-empty surface display. Fixed (sodium azide treated) and unfixed diabetic B cells maintained at 4°C dramatically bound exogenous peptide; eliminating differences in

surface turnover of MHC class II as the sole reason for the abundance of peptide MHC class II-empty products on the cell surface.

The amount of HA peptide or IgCκ MHC class II bound peptide, represented as a percent change from background fluorescence after peptide exposure confirmed the dramatic difference in exogenous peptide binding of MHC class II between diabetic twin and non-diabetic twin cell lines, regardless of the individual MHC class II haplotypes of any twin set.

The ability of surface displayed MHC class II on diabetic twin cell lines to bind exogenous peptide is indicative of abnormal intracellular peptide loading. Once again, this observed difference in relative occupancy of surface MHC class II is not due to MHC class II allelic influences since comparisons were between MHC matched twin cell sets for the extended haplotypes of all MHC class II gene products.

EXAMPLE 2.4: Cell Surface Arrival, Turnover, and Half-Life of MHC Class II

The capability of APCs to educate and to stimulate (positively or negatively) T cell activity is also dependent on the maintenance and stability of MHC class II-peptide surface display. To demonstrate further abnormal MHC class II display phenotypes indicative of Type I diabetes, MHC class II cell surface arrival and turnover was examined in matched diabetic twin and non-diabetic twin B cell lines using radioactive monitoring techniques and surface display half-life assays.

EXAMPLE 2.4.1: Pulse-Chase Monitoring of MHC Class II Transport and Turnover

Pulse-chase techniques well known in the art were used to examine the rate of arrival and persistence of MHC class II surface display. Cell-surface biotinylation combined with radioactive monitoring of total rates of MHC class II synthesis were used to directly assess MHC class II assembly (Harter & Mellman, 1992; Hunziker & Mellman, 1991; Pierre et al., 1997).

Approximately 3×10^7 cells per test sample were pulse-labeled with 1 mCi [S^{35}]methionine translabel (Dupont, Boston, MA) in 5 ml of methionine-free RPMI 1640 for 30 min at 37°C and chase for time; 0, 30, 60, 120 and 240 min at 27°C in complete RPMI 1640 medium with a 15-fold excess of cold methionine.

Prior to cell lysis, cells were suspended in 1 ml Hanks, and incubated with 3.0 mg/ml of N-hydroxy succinamide-S-S-biotin (NHS-SS-biotin, Pierce, St. Louis, MO) for 3 minutes on ice as described by Amigorena et al. (1995). Because NHS-SS-biotin is membrane-impermeable, the reagent labels only surface structures.

Biotinylated and radiolabeled cells were lysed for 30 minutes on ice in 10 mM Tris, pH 7.4 (TBS) contains 150 mM NaCl, 1% Triton™-100, 0.5 mM PMSF, 0.1 nM TLCK. Lysates were precleared by 50% (v/v) protein A-SEPHAROSE (Pharmacia, Piscataway, NJ) overnight. Precleared lysates were immunoprecipitated with anti-MHC class II DR monoclonal antibody (L243) and protein A-SEPHAROSE. Biotinylated proteins were eluted from the beads in 100 µl PBS containing 2% SDS. A 20 µl aliquot (representative of total MHC class II) was transferred onto a SDS-PAGE gel for analysis.

To determine surface MHC class II density, an 80 ul aliquot was diluted into 1 ml PBS with 1% TX-100. Diluted lysates were precipitated with Streptavidin-Agarose beads (Pierce, St. Louis, MO) for 2 hr at room temperature, then washed three times with precipitate buffer and transferred to SDS sample buffer. Immunoprecipitates were analyzed on 12% SDS-PAGE gel and quantified with NIH image 1.61/Fat program.

The results are presented in Figure 2. Although the overall rate of MHC class II synthesis in diabetic twin cells (DM) was comparable to matched non-diabetic twin cells (T), diabetic twin cells demonstrated an abnormal (rapid) rate of delivery of MHC class II to the cell surface. MHC class II of diabetic twin cells was detected on cell surface by 1 hour, and was fully present by 2 hours. In contrast, matched non-diabetic twin cells (T) and control cells (Con) recorded little or no MHC class II surface display at 2 hours.

Figure 2 further illustrates another abnormal MHC class II display in diabetic cells compared to non-diabetic cells, i.e., the MHC class II display in diabetic cells exhibits rapid surface turnover. At 3 hours, the time at which non-diabetic twin and control cells have not even demonstrated maximal MHC class II surface display, MHC class II display for diabetic twin cells has started to decline.

Abnormally rapid delivery and turnover of MHC class II to the cell surface of diabetic twin cells, compared to matched non-diabetic twin and control cells, was consistent in all test trials. These results confirm data from other pulse-chase experiments, which demonstrate rapid MHC class II-Ii transport in diabetic cells (see Example 3.2), and support the model that MHC class II molecules in diabetic cells move rapidly through the cell, exiting the ER rapidly and are either transported through the Golgi complex rapidly (resulting in abnormal glycosylation), or by-pass the Golgi complex altogether, reaching the cell surface rapidly and concomitantly turning-over rapidly.

EXAMPLE 2.4.2: Half-life Determination of MHC Class II-Peptide Display

Earlier examples demonstrate that despite apparently normal MHC class II surface density observed in diabetic cells as compared to non-diabetic cells, MHC class II display in diabetic cells involves abnormally rapid transport to the cell surface and that MHC class II display in diabetic cells is predominantly peptide empty. Earlier presented data also indicate that normal overall MHC class II surface density in diabetic cells, despite abnormally rapid MHC class II transport, is due to a concomitant abnormally rapid turnover of displayed MHC class II. MHC class II surface turnover rates for diabetic twin cells compared to non-diabetic and control cells were analyzed using alternative techniques known in the art for quantifying MHC class II display half-life (e.g., see Amigorena, 1995; Pierre & Mellman, 1998).

To determine MHC class II-peptide display half-life ($t_{1/2}$) in diabetic twin, non-diabetic twin and control cells, the persistence of either peptide-filled MHC class II DR or DQ complexes was assessed by measuring the stability (via pulse labeling with N-terminal biotinylated peptides) of bound MHC class II-DR peptides (described earlier) or bound MHC class II-DQ peptides (SGPLKAEIAQRLEY; SEQ ID NO:3, known to bind MHC-DQ alleles with varying affinity) on the surface. It has been reported that peptide dissociation is extremely low and non-stoichiometric at saturating peptide concentrations. MHC class II-peptide surface displays are viewed as irreversible. Monitoring the change of labeled peptide (by immunofluorescence) over time, therefore, is indicative of change (i.e., turnover) in the MHC class II-peptide display (not mere dissociation of bound peptides from MHC class II; see Lanzavecchia et al., 1992; Buus et al., 1986)

Cells were preincubated with 50 μM of biotinylated synthetic peptide for 4 hr at 37°C, and washed three times. Following the four-hour preincubation, cells were incubated in media containing 500 μM of cold competitive peptide (non-biotinylated peptide) for 0, 12, 24, 36, and 48 hours at 37°C. At the indicated time periods, cells were subjected to FACS analysis. Remaining MHC class II-peptide surface complexes, quantified by flow cytometry, is indicative of MHC class II-peptide display.

The results are presented in Figure 3 as mean fluorescence percent over time. Although non-diabetic twin cell fluorescence was initially lower than that of the control cells, both tracked similar fluorescent decay over time, indicating similar internal MHC class II processing and transport. In contrast, mean fluorescence for diabetic twin cells was initially higher (due to peptide feeding of MHC class II-empty surface displays), and was followed by an abnormally rapid decrease in surface immunofluorescence, indicating a rapid turnover of MHC class II-

peptide surface display, and an abnormally short half-life of MHC class II-peptide display (Table 3).

The data shown in Figure 3, represent the comparative results from diabetic discordant twin set #3 and a matched MHC class II DR control. Similar rapid half-live profiles for four additional diabetic twins were recorded for DR bound human IgCκ peptide (data not shown); further indicating a lack of basal MHC class II-peptide surface display for diabetic cells at time zero, and rapid turn-over of peptide filled MHC class II complexes on the diabetic B cells. In addition, half-lives of labeled peptides were unaffected by dose response incubations of varying labeled and competitive peptides indicating saturating concentrations (data not shown). Filling empty peptide grooves of surface MHC class II diabetic structures, therefore, did not restore internal MHC class II assembly and transport to normal.

TABLE 3. MHC Class II-Peptide Presentation (Intracellular and Surface) Half-Life in Discordant Type I Diabetes Cell Lines

Discordant Diabetic Twin Set	$t_{1/2}$	
	Diabetic Twin	Non-diabetic Twin
#1	2.88	5.84
#2	3.72	5.23
#3	3.98	5.04
#4	2.97	5.13

Data represent triplicate samples analyzed on the same day with cells grown under identical conditions. Triplicate samples demonstrated less than 5% variability between assay tubes. The assay was performed on four separate occasions with identical trends. Half-life calculations were obtained by linear regression analysis.

EXAMPLE 3.0: Intracellular Assembly & Transport of MHC Class II Complexes

To characterize abnormal MHC class II phenotypes associated with Type I diabetes, a variety of intracellular MHC class II presentation phenotypes (assembly and transport phenotypes) were examined in EBV transformed B cell lines (as described in Example 1 above). Among the MHC class II phenotypes examined were: MHC class II-Ii glycosylation profiles, MHC class II transport from the endoplasmic reticulum, and MHC class II dimer formation.

As one method of analyzing, comparing, and characterizing MHC class II assembly and transport of diabetic twin cells and their matched non-diabetic twin cells, serial 4 hour pulse-chase experimental protocols well known in the art were employed. Immunoassays were performed with a variety of MHC class II antibodies.

For pulse-chase experiments, 5×10^7 of cells were pulse-labeled with 2 mCi of [S^{35}]methionine translabelled in 15 ml methionine-free RPMI 1640 for 30 minutes at 37°C and chased in complete RPMI 1640 with 15-fold excess of cold methionine at set time intervals. Radiolabeled cells were lysed at 4°C in 10 mM Tris, pH 7.4 containing 150 mM NaCl, 1% TRITON-100, 0.5 mM PMSF, 0.1 nM TLCK.

For MHC class II-peptide detection, cells were pulse-labeled with [S^{35}]methionine for 1.5 hr, chased for 4 hr or 15 hr, and lysed as described above. Precleared lysates were precipitated with MHC anti-class II or Ii MAb (provided by Dr. Peter Cresswell, Yale University) and washed three times before protein elution from beads by SDS loading buffer.

For double immunoprecipitation procedures, the first antibody precipitates were added to SDS loading buffer (containing 100 mM Tris-Cl, pH 6.8, 2% SDS, 2% Glycerol) and incubated at room temperature for 2 hr. Eluted proteins were precipitated again by a second related MAb to MHC class II on the invariant chain.

For EndoH resistance procedures, the precipitates were eluted in 100 µl EndoH digestion buffer (0.1 M sodium phosphate, 0.5% SDS, 0.1% NaNs, pH 6.5) at 100°C for 6 min. EndoH buffer eluted protein was incubated with (or without) 2 milliunits of EndoH for 16 hr at 37°C. The protein was analyzed on 12.5% SDS PAGE gel under reducing or non-reducing conditions. For MHC class II-peptide formation analysis, half of the samples were boiled at 100°C for 2 minutes and the other half were maintained at room temperature. Both treatment trials were analyzed by SDS-PAGE.

EXAMPLE 3.1: MHC Class II-Ii Glycosylation

To characterize glycosylation patterns of molecules exported out of the ER, portions of immunoprecipitate were treated with EndoH at specified time intervals. EndoH is an enzyme that cleaves early carbohydrate additions of MHC class II-Ii complexes prior to Golgi compartment processing (Kornfeld, 1988). Detection of a molecular weight shift (i.e., reduction in MW) in EndoH-treated MHC class II-Ii is due to enzymatic cleavage of the N-linked carbohydrate molecule (EndoH sensitivity), and indicates the MHC class II-Ii complex is still located in the ER. Pulse-chase measurements (as described earlier) of EndoH sensitivity permit the glycosylation patterns, and rate and route of intracellular MHC class II transport (example 3.2 below) to be quantified.

Figure 4 is representative of pulse chase experimental results characteristic of discordant diabetic twin set trials. Four paired identical twin sets were studied, producing identical temporal profiles. At time equal 0 or 30 min. of pulse-chase, both diabetic twin and non-diabetic twins

cells exhibited EndoH sensitivity; indicating that the MHC class II-invariant chain complexes had not yet transversed the Golgi component, as expected. Notably, however, diabetic twin cells exhibited minimal Ii chain associations before EndoH treatment at these early time intervals. Even more significant, there was a specific deficiency of MHC class II binding to Ii-p35 (the site indicated by arrowheads in Fig. 4) in the diabetic twin sample compared to the non-diabetic twin sample. In addition, the diabetic twin exhibited minimal band heterogeneity after EndoH treatment at these early time periods (the region indicated by bars in Fig. 4). MHC class II-Ii band heterogeneity was observed in the non-diabetic twin and is indicative of multiple post-translational modifications representative of complex carbohydrate additions.

The MHC class II carbohydrate marker data from these early time intervals indicate that diabetic twin cells contain abnormally diminished MHC class II-invariant carbohydrate additions, which usually signals abnormally shortened transport times, abnormal intracellular routing, or abnormal Ii chain associations.

EXAMPLE 3.2: MHC Class II-Ii Transport from the ER

To analyze further MHC class II-Ii transport out of the Golgi in diabetic twin versus non-diabetic twin cells, the pulse-chase trials of EndoH treated and untreated samples described in examples 3.0 and 3.1 were continued over several hours. Figure 4 is representative of pulse chase experimental results characteristic of discordant diabetic twin set trials. Once again, the four paired identical twins studied produced identical temporal profiles.

Biochemical phenotypes of the MHC class II-Ii chain assembly in diabetic twin compared to non-diabetic twin cells continued to exhibit distinguishing characteristics at additional monitoring time intervals of the pulse chase experiment. MHC class II-Ii complexes in diabetic twin cells exhibited partial to total EndoH resistance at time equal 120 to 180 min. of pulse-chase, and complete resistance by 240 min. In contrast, non-diabetic twin cells showed only partial EndoH resistance with continuing band heterogeneity in complex carbohydrate addition up to 240 min. of pulse-chase. In fact, non-diabetic twin and control cells did not exhibit total EndoH resistance until 360-480 min. and 600 min., respectively (data not shown).

In summary, biochemical assessment of MHC class II-Ii assembly and transport in pulse chase experiments demonstrate diabetic cells have impaired glycosylation and rapid ER exit of the complex followed by rapid EndoH resistance compared to matched non-diabetic twin and control cells. This pattern was unequivocal among all diabetic twin , non-diabetic twin and control cells studied.

EXAMPLE 3.3: MHC Class II Dimer Assembly

MHC class II-empty dimers are generally unstable (Nelson, 1993; Sadegh-Nasseri, 1991), while tightly bound MHC class II-peptide complexes are quite stable. MHC class II-peptide complexes form within the endosomal/lysomal compartments of the endocytic pathway. Cells exhibiting abnormal MHC class II routing and transport (as exemplified and phenotyped in the previous examples), producing a disproportionate amount of MHC class II empty molecules, would also exhibit an abnormal MHC class II dimer stability phenotype.

To demonstrate that MHC class II stability in diabetic twin cells exhibit a characteristically abnormal phenotype compared to their paired non-diabetic twin cells, cell lines from each twin set were pulse labeled for 30 minutes, chased for 4 hours or 15 hours, and MHC class II immunoprecipitations were SDS-PAGE analyzed with and without boiling at 95°C. Monoclonal antibody 9.3F10 (described earlier in Example 2.1) or MAb DA6.147(Guy, 1982) was used on cellular lysates.

Figure 5 is illustrative of the results from SDS-PAGE analysis for the four discordant twin pairs examined. MHC class II compact dimers formed at 4 hours and were present in the non-diabetic twin cells, but were less formed on diabetic twin cells after 4 hours of chase. Small amounts of compact MHC class II dimer formation were possible in diabetic twin B cell lines but time course experiments documented the conversion to compact dimers was significantly delayed and only evident at maximal intensity in diabetic B cell lines after a chase of 15 hrs. Even at hour fifteen, diabetic twin cells exhibited significant amounts of MHC class II subunits (α and β) lacking compact dimer formation.

That MHC class II molecules of distinct isotype, allele and species origin exhibit marked differences in the proportion of stable and unstable dimers generated late in synthesis is well known to practitioners in the art (Germain & Hendrix, 1991; Gorga, 1987). Random control cell lines lacking at risk MHC class II haplotypes displayed greater dimer formation than non-diabetic twin cells at 4 hours of chase supporting the prior reports on the genetic influences of diabetic MHC class II haplotypes (data not shown). Delayed SDS-PAGE stability has been correlated with short MHC class II half-life (Kumar et al., 1993).

These results, however, represent the first report of MHC class II dimer formation phenotypes as an epigenetic phenomenon, and confirm, for the first time, that an abnormal MHC class II dimer phenotype is a unique and characteristic marker for a disease or disorder associated with MHC class II presentation. Abnormally delayed MHC class II dimer formation is indicative of this form of Type I diabetes.

EXAMPLE 4.0: Ii-p35 Isoform Effects on MHC Class II Transport

Because MHC class II heterodimer formation phenotypes may be influenced by the chaperone machinery involved in MHC class II presentation (Lotteau, 1990; Warmerdam, 1996), peptide profiles of one of the predominant Ii chain isoforms, Ii-p35, was examined in EBV transformed cell lines (as described in Example 1 above).

EXAMPLE 4.1: Invariant Chain Isoform Expression in Discordant Twin Cells

Western blot analyses were performed on discordant twin cell lines to demonstrate the disease-specific phenotype of abnormally reduced cellular levels of Ii-p35 in diabetic twin cells compared to matched non-diabetic twin cells.

Western blot analysis was performed following protocols well known in the art. For each trial, 2×10^7 cells were harvested and lysed with 500 µl of lysis buffer A (1% NP-40, 10 mM Tris HCl, 0.15 NaCl, 0.2 mM EDTA, 0.02% NaN3, 100µg/ml PMSF, 1 µg/ml aprotinin, 1 µg/ml leupeptin). Protein concentration was determined by BCA protein assay kit (Pierce, Rockford, Illinois). Equal amounts of protein samples were subjected to 12.5% SDS-PAGE treatment, and transferred to a PVDF membrane in methanol transfer buffer. Membrane filters were rinsed twice with 0.1% TWEEN-20 in PBS, and blocked with 5% non-fat dry milk.

Human anti-Ii chain polyclonal antibodies to Ii-p35 and Ii-p41 isoforms were used for immunoprecipitation (provided by Dr. Peter Cresswell, Yale University, New Haven, CT). A control Polyclonal anti-HC3, directed to a conserved human T20S proteasome subunit (Affiniti Research Products, Ltd., Mamhead, Exeter, United Kingdom), was used as a control. Antibodies were diluted from 1:5,000 to 1:10,000 and incubated with the membrane filter for 60 min., washed 3X for 5 min., and incubated with 1:20,000 dilute control antibody. The washed filter was treated with substrate (ECL plus, Amersham, Piscatway, NJ) for 5 min. The film was exposed for 2-10 min.

Figure 6 depicts Ii-p35 expression levels and patterns in matched diabetic and non-diabetic twin cells. For each discordant twin cell line, overall invariant chain expression levels were normal, and Ii-p41 isoform levels were comparable. In addition, protein levels of control proteasome subunit HC3 were equivalent within the discordant twin sets.

In stark contrast, however, Ii-p35 isoform levels were reduced 40-50% in the diabetic twins. This abnormally reduced Ii-p35 isoform level was specific for the diabetic twin. Because overall invariant chain expression levels were normal, and Ii-p35 and Ii-p33 isoforms are made from alternate mRNA transcripts of the same locus, these data suggest that diabetic twin cells exhibit reduced translation of the Ii-p35 isoform. Alternatively (although less likely), an increase

in Ii-p35 degradation could also account for decreased static levels of MHC class II-p35 in the diabetic twin cells. Regardless of the underlying mechanism, these results demonstrate that abnormally reduced levels of Ii-p35 isoform in cellular lysates is indicative of Type I diabetes.

EXAMPLE 4.2: Full Length Sequence of Ii Chain cDNA and gDNA

To refute the unlikely hypothesis that somatic mutation in the promoter, coding region, or early introns of the Ii locus, sufficient to alter translation, is responsible for the characteristic phenotypic distinctions observed between diabetic and non-diabetic cells, full length invariant chain cDNA and gDNA from four twin sets discordant for diabetics and from one random non-diabetic control were sequenced and compared.

The cDNA and promoter sequences were identical and in complete agreement with published invariant chain sequences (data not shown; see, Doyle, 1990).

EXAMPLE 4.3: Alteration of MHC Class II Presentation by Stable Transfection with Ii-p35

To demonstrate the functional role of Ii-p35 in the onset and progression of MHC class II presentation-related diseases and how alteration of abnormal Ii-p35 cellular levels to within normal ranges effectively restores MHC class II presentation, diabetic twin and non-diabetic twin cells were stably transfected with an Ii-p35 expression vector.

All transfection experiments reported here were carried out using an Ii-p35 cDNA expression construct. For the Ii chain vector to express Ii-p35 exclusively, the second AUG translational start site of the Ii-p33/35 transcript was mutated (see Strubin, 1986). The mutant cDNA was operably spliced (at BamH1 sites) from its parent vector (Sp64) to a vector specifically engineered for high level expression of cDNA in mammalian cells (i.e., the pBABE retroviral vector; Morgenstern and Land, 1990). Human cells from paired twin sets were stably transfected with Ii-p35 cDNA in expression vector pBABE/p33ATG1 (parallel discordant twin cell samples were transfected with empty vector as controls).

Transfection was performed by electroporation, using techniques well known in the art. Briefly 1×10^7 cells with 30μg of purified pBABE/p35 vector were placed on ice for 5 minutes and electroporated at 220V, 960 Ω in a 0.4 cm cuvette. Transfected cells were grown for 2 days without selection followed by continuous maintenance in 0.25 μg/ml of puromycin for over 2 months. For each cell line, at least 3 stable and independently derived cell lines were established from transfections performed on various days. Ii-p35 expression levels were analyzed using standard Western blot techniques known in the art (see Example 4.1).

Figure 7 depicts the Western blot analysis for twin set #4 and is representative of all twin sets analyzed. Overall levels of total invariant chain levels for Ii-p35 transfected diabetic cells were comparable to transfected non-diabetic cells (i.e., were within normal limits). In addition, twin cells transfected with Ii-p35 appeared to maintain stable ratios of the isoform variants.

To quantify the alteration (i.e., correction to normal levels) of MHC class II presentation in diabetic twin cells transfected with the Ii-p35 expression vector, peptide occupancy of endogenously filled surface MHC class II structures on diabetic cells was compared to that of non-diabetic Ii-p35 transfected cell lines.

Using biotinylated HA peptide(307-319) stably transfected diabetic and non-diabetic twin cells were exposed for four hours followed by flow cytometry for the test antigens at saturating conditions after development with fluorescinated avidin (see Example 2.3).

Consistent with results presented earlier, untransfected diabetic cells demonstrated an abundance of peptide MHC class II-empty structure by the ability to load more exogenously added peptide (Fig. 8A). Ii-p35 transfected diabetic twin cells (DMp35) decreased fluorescent peptide binding compared to the untransfected diabetic cell line (DM), indicating improved intracellular peptide loading of MHC class II (Fig. 8B). On the log scale of fluorescence, the transfected diabetic cell line (DM p35) bound reduced exogenous added peptide comparable to the non-diabetic twin cell line (Fig. 8C), indicating an alteration of endogenous peptide loading in diabetic cells to levels comparable to the unaffected twin (T) cell line (i.e., normal endogenous peptide loading). Finally, Ii-p35 transfected non-diabetic twin cell (T p35) exogenous peptide loading profiles were not altered from that exhibited by non Ii-p35 transfected non-diabetic twin cells, demonstrating that a critical amount of Ii-p35 protein is essential for MHC class II assembly but possible overexpression of Ii-p35 does not alter MHC class II peptide loading processes to produce an overabundance of endogenously filled peptide MHC class II display. In fact, diabetic cells expressing Ii-p35 vector (DMp35) bound peptide in a normally restored manner similar to twin cells or twin cells stably transfected with Ii-p35 (Fig. 8D).

A third biochemical parameter of altered MHC class II processing was directed to the diabetic twin phenotype of rapid MHC class II arrival and surface turnover (see Example 2.4.1 above). To demonstrate the effectiveness of altering Ii-p35 protein levels in diabetic cells, pulse-chase studies involving surface biotinylation combined with radioactive monitoring of total rates of MHC class II synthesis were performed comparing Ii-p35 untransfected (DM) and Ii-p35 transfected (DMp35)cell lines.

The results are presented in Figure 9. Once again, consistent with results presented earlier, untransfected diabetic cells demonstrated extremely rapid delivery to MHC class II to the

surface and rapid surface turnover compared to non-diabetic twin cells. Ii-p35 transfected diabetic twin cells, however, exhibited MHC class II surface arrival and turnover identical to the matched non-diabetic twin cell profile.

These results support the critical functional role of deficient Ii-p35 in diabetic cells conferring dramatically abnormal phenotypes in MHC class II presentation (i.e., assembly, transport and display), demonstrate that transfection with an Ii-p35 expression vector effectively alters MHC class II presentation phenotypes in diabetic cells to that of normal (non-diabetic) MHC class II presentation, and demonstrate that treatment of supplemental Ii-p35 effectively corrects MHC class II display of endogenous peptides in diabetic cells to normal parameters.

EXAMPLE 5.0: MHC Class II-CLIP Binding Affinity

The present discordant diabetic twin studies demonstrate that inadequate cellular levels of Ii-p35 result in a multitude of abnormal MHC class II presentation phenotypes, which are characteristic to diabetic cells and independent of genetic influences. MHC class II allelic factors may still exert effects on MHC class II presentation, however, specifically as they affect Ii chain complexation and processing in the endosome. Such genetically based phenotypes, which would not appear disparate in identical twin analyses, may represent direct allelic influences conferred by at-risk MHC class II genotypes.

To examine the relationship between differing at-risk MHC class II genotypes and MHC class II-Ii binding affinity, *in vitro* competitive binding and dissociation assays of various human MHC (HLA) class II genotypes to synthetic CLIP versus radiolabeled human CLIP peptides were performed.

Purified HLA class II peptides from 5 allelic variants were purchased or purified from cell lines obtained commercially (Human Biologic Data Interchange, Philadelphia, PA; and Coriel Institute, Camden, NJ). The five HLA genotypes confer statistically different susceptibility to (i.e., at-risk for) diabetes.

A fragment of the human invariant chain, which binds to the HLA class II groove (CLIP; AA 81-104) was synthesized (Pharmingen, San Diego, CA) and labeled with ^{125}I prior to purification. To measure labeled CLIP binding, each allelic HLA class II peptide was added at a concentration of ~200 nM, and incubated with a saturating concentration of labeled CLIP for 48 hr. The percentage bound radioactive was measured after the complexes had been column purified using standard protocols.

Relative binding affinities are presented in Table 4. The two high risk MHC class II alleles exhibited the lowest binding affinity for the CLIP fragment, while low risk and protective

MHC class II alleles exhibited the highest binding affinity for the CLIP fragment (the one MHC class II allele with neutral statistical risk exhibited intermediate affinity for CLIP).

These results demonstrate an inverse relationship between at-risk MHC class II genotype for diabetes and CLIP fragment binding, and suggest that MHC class II alleles confer intrinsic biochemical properties, which affect endosomal/lysosomal compartment processing (MHC class II-Ii complex formation and retention); and ultimately peptide loading.

TABLE 4. Relative Binding of HLA Class II Alleles to Human Ii Chain Fragment (81-104)

HLA class II genotype	At-Risk for Diabetes	Affinity for Invariant Chain* ($\times 10^{-4}$)
DQB1 0302	High	2.7
DRB1 0401	High	7.1
DRB1 0501	Neutral	7.5
DRB1 0404	Low	25.0
DQB1 0602	Protective	311.6

*High affinity indicates tight Ii chain binding to MHC class II proteins.

Accelerated degradation of the MHC class II-Ii complex is due to inadequate glycosylation, stemming from a deficiency of Ii-p35, and hence accelerating the kinetics of ACE degradation. These effects may be exacerbated by an allelic variant of MHC class II that poorly binds the Ii chain (this allelic influence for invariant chain dissociation is demonstrated in Table 2 where a 200-fold difference in Ii chain affinity is observed between a given class II allelic variant and a peptide binding CLIP fragment of the invariant chain). These genetic coding influences, both in the MHC and non-MHC regions, still only contribute less than an estimated 40-50% influence on disease expression in humans based on identical twins studies. The biochemical correction of MHC class II presentation with Ii-p35 transfection accounts for an important epigenetic component, which has a determining effect on ultimate disease manifestation.

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All of the publications cited herein are hereby incorporated by reference in their entirety.

CLAIMS:

1. A method for detecting abnormal MHC class II presentation in a cell comprising:
   a) obtaining a sample comprising an antigen presenting cell, an antigen presenting cell extract, or an antigen presenting cell lysate; and
   b) detecting an abnormal amount of Ii chain peptide in said sample.

2. The method of claim 1 wherein said Ii chain is an Ii isoform containing an N-terminal ER retention signal.

3. The method of claim 2 wherein said Ii isoform is selected from the group consisting of Ii-p35 and Ii-p43.

4. The method of claim 3 wherein said Ii isoform is Ii-p35.

5. The method of claim 4 wherein said abnormal amount of Ii-p35 is an inadequate amount of Ii-p35.

6. A method for testing a mammal for predisposition to develop a disease associated with abnormal MHC class II presentation comprising:
   a) obtaining a sample from said mammal comprising an antigen presenting cell, an antigen presenting cell extract, or an antigen presenting cell lysate; and
   b) detecting an abnormal MHC class II presentation phenotype in said sample, wherein said abnormal MHC class II presentation phenotype is indicative of said predisposition.

7. The method of claim 6 wherein said MHC class II presentation phenotype is a MHC class II assembly phenotype.

8. The method of claim 7 wherein said MHC class II assembly phenotype is a MHC class II-Ii glycosylation phenotype.

9. The method of claim 8 wherein said MHC class II-Ii glycosylation phenotype exhibits abnormally diminished glycosylation.

10. The method of claim 7 wherein said MHC class II assembly phenotype is MHC class II dimer stability.

11. The method of claim 10 wherein said MHC class II dimer stability is abnormally delayed.

12. The method of claim 7 wherein said MHC class II assembly phenotype is endosomal degradation.

13. The method of claim 12 wherein said MHC class II endosomal degradation is abnormally rapid.

14. The method of claim 7 wherein said MHC class II assembly phenotype is MHC class II affinity for an Ii chain peptide.

15. The method of claim 7 wherein said MHC class II assembly phenotype is an Ii chain peptide phenotype.

16. The method of claim 15 wherein said Ii chain peptide phenotype is an Ii isoform phenotype, wherein said Ii isoform contains an N-terminal ER retention signal.

17. The method of claim 16 wherein said Ii isoform is selected from the group consisting of Ii-p35 and Ii-p43.

18. The method of claim 17 wherein said Ii isoform is Ii-p35.

19. The method of claim 18 wherein said Ii isoform phenotype is an abnormally low amount of Ii-p35.

20. The method of claim 6 wherein said MHC class II presentation phenotype is a MHC class II transport phenotype.

21. The method of claim 20 wherein the rate of MHC class II transport from the ER is measured.

22. The method of claim 21 wherein an abnormally rapid rate of MHC class II transport from the ER indicates said predisposition.

23. The method of claim 6 wherein said MHC class II presentation phenotype is a MHC class II display phenotype.

24. The method of claim 23 wherein said MHC class II display phenotype is a MHC class II-empty display phenotype.

25. The method of claim 24 wherein abnormally abundant MHC class II-empty display is indicative of said predisposition.

26. The method of claim 23 wherein said MHC class II display phenotype is a MHC class II-CLIP display phenotype.

27. The method of claim 26 wherein said MHC class II-CLIP display phenotype exhibits abnormally reduced MHC class II-CLIP display.

28. The method of claim 26 wherein said MHC class II display phenotype is measured by determining the rate of arrival of MHC class II at the cell surface.

29. The method of claim 28 wherein determination of an abnormally rapid rate of arrival of MHC class II at the cell surface is indicative of said predisposition.

30. The method of claim 23 wherein said MHC class II display phenotype is determined by measuring MHC class II surface turnover.

31. The method of claim 30 wherein determination that said MHC class II surface turnover is abnormally rapid indicates said predisposition.

32. The method of claim 23 wherein said MHC class II display phenotype is determined by measuring MHC class II half-life.

33. The method of claim 32 wherein determination that said MHC class II half-life is abnormally short indicates said predisposition.

34. A treated autologous mammalian antigen presenting cell suitable for treatment of a disease associated with abnormal MHC class II presentation, said cell exhibiting abnormal MHC class II antigen presentation, wherein said cell has been treated so as to reverse said abnormal MHC class II antigen presentation such that said cell exhibits normal MHC class II antigen display.

35. The treated cell of claim 34 wherein said cell exhibits, prior to treatment, at least one abnormal MHC class II presentation phenotype selected from the group consisting of: reduced MHC class II-Ii chain glycosylation; reduced MHC class II-peptide loaded display; decreased MHC class II-CLIP fragment display; increased MHC class II-empty display; delayed MHC class II heterodimer stability; reduced Ii chain isoform possessing the N-terminal ER retention signal; abnormal cellular level of Ii-p35 containing Ii chain trimer; reduced cellular level of Ii-p35 isoform; reduced cellular level of Ii-p45 isoform; increased rate of MHC class II complex exit from the ER or other intracellular compartment; abnormally high rate of MHC class II complex exit from the Golgi complex; MHC class II bypass of the Golgi complex; increased rate of MHC class II complex endosomal degradation; increased rate of MHC class II complex surface arrival; increased rate of MHC class II complex surface turnover; and decreased MHC class II half-life;
wherein said cell has been treated to reverse said at least one abnormal MHC class II presentation phenotype.

36. The treated cell of claim 35 wherein said cell exhibits, prior to treatment, abnormally low expression of Ii-p35 or Ii-p43 or low expression of both.

37. The treated cell of claim 36 wherein said cell is treated by transfecting said cell with an expression vector effective for increasing expression within said cell of Ii-p35 or Ii-p43 or both.

38. A pharmaceutical composition for treatment of a mammal predisposed to develop Type I diabetes comprising a treated cell according to claim 34 and a pharmaceutically acceptable carrier.

39. A method for altering MHC class II antigen presentation of a cell comprising:
    a) providing an antigen presenting cell; and
    b) altering a MHC class II presentation phenotype in said antigen presenting cell.

40. The method of claim 39 wherein said MHC class II presentation phenotype is a MHC class II assembly phenotype.

41. The method of claim 40 wherein said MHC class II assembly phenotype comprises abnormally low MHC class II-Ii glycosylation.

42. The method of claim 41 wherein said cell is treated so as to increase MHC class II and/or Ii peptide glycosylation.

43. The method of claim 40 wherein said MHC class II assembly phenotype is MHC class II dimer stability.

44. The method of claim 43 wherein said cell is treated so as to increase MHC class II dimer stability.

45. The method of claim 40 wherein said MHC class II assembly phenotype is endosomal degradation.

46. The method of claim 45 wherein said cell is treated so as to inhibit MHC class II endosomal degradation.

47. The method of claim 40 wherein said MHC class II assembly phenotype is an Ii chain peptide phenotype.

48. The method of claim 47 wherein said Ii chain peptide phenotype comprises abnormally low amounts of an Ii isoform having an N-terminal ER retention signal peptide.

49. The method of claim 48 wherein said Ii isoform is selected from the group consisting of Ii-p35 and Ii-p43.

50. The method of claim 49 wherein said Ii isoform is Ii-p35.

51. The method of claim 48 wherein said Ii chain peptide phenotype is altered to increase the amount of said Ii isoform produced by said cell.

52. The method of claim 51 wherein said cell is transfected with an expression vector suitable for production of Ii isoform Ii-p35 in said cell.

53. The method of claim 39 wherein said MHC class II presentation phenotype is a MHC class II transport phenotype.

54. The method of claim 39 wherein said MHC class II presentation phenotype is a MHC class II display phenotype.

55. The method of claim 54 wherein said MHC class II display phenotype comprises MHC class II-empty display at the surface of the cell.

56. The method of claim 54 wherein said MHC class II display phenotype comprises an abnormally increased rate of arrival of MHC class II to the cell surface.

57. The method of claim 54 wherein said MHC class II display phenotype comprises an abnormally high rate of MHC class II surface turnover.

58. The method of claim 54 wherein said MHC class II display phenotype comprises an abnormally shortened MHC class II half-life.

59. The method of claim 39, wherein said MHC class II presentation phenotype is altered by providing an effective amount of isolated Ii chain peptide, said isolated Ii chain peptide

comprising an Ii isoform, wherein said Ii isoform contains an N-terminal ER retention signal peptide.

60. The method of claim 59 wherein said Ii isoform is selected from the group consisting of Ii-p35 and Ii-p43.

61. The method of claim 60 wherein said Ii isoform is Ii-p35.

62. The method of claim 59 wherein said Ii chain peptide is an Ii trimer containing at least one Ii isoform, wherein said Ii isoform contains an N-terminal ER retention signal.

63. The method of claim 62 wherein said Ii isoform is Ii-p35.

64. The method of claim 39, wherein said MHC class II presentation phenotype is altered by providing an effective amount of isolated MHC class II molecules, said isolated MHC class II molecules having a higher binding affinity to an Ii chain peptide compared to MHC class II molecules normally present in said antigen presenting cell.

65. The method of claim 39, wherein said MHC class II presentation phenotype is altered by inhibiting an enzyme normally present in said antigen presenting cell, wherein said enzyme cleaves Ii chain peptides.

66. The method of claim 39, wherein said MHC class II presentation phenotype is altered by stimulating phosphorylation of Ii chain peptides normally present in said antigen presenting cell.

67. The method of claim 39, wherein said MHC class II presentation phenotype is altered by providing exogenous peptides to said antigen presenting cell, wherein said exogenous peptides bind to MHC class II-empty complexes.

68. A method for altering MHC class II presentation of a cell comprising:

a) providing an antigen presenting cell; and

b) transfecting said antigen presenting cell with an expression vector, wherein said expression vector operably expresses an Ii chain isoform.

69. The method of claim 68 wherein said Ii chain is an Ii isoform containing an N-terminal ER retention signal.

70. The method of claim 69 wherein said Ii isoform is selected from the group consisting of Ii-p35 and Ii-p43.

71. The method of claim 70 wherein said Ii isoform is Ii-p35.

* * * * *

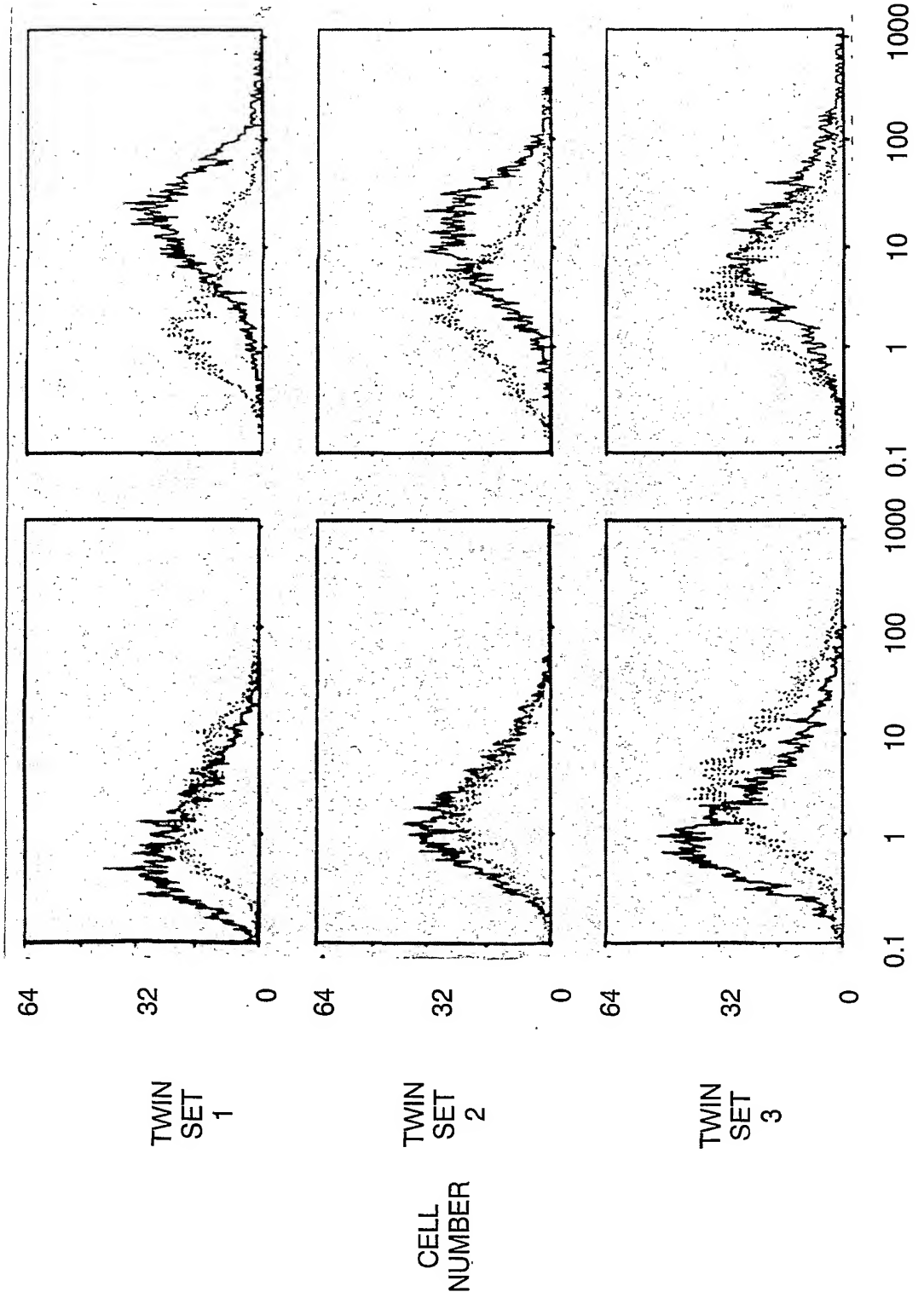
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FIG. 1A

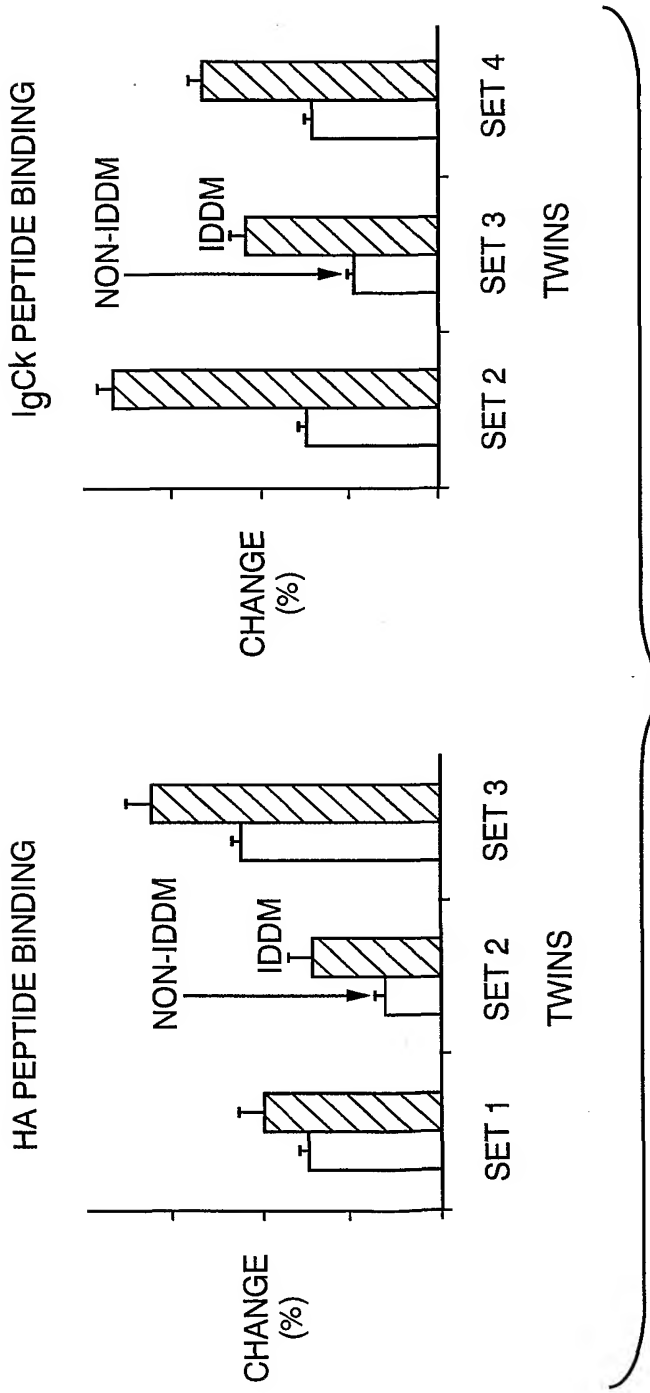
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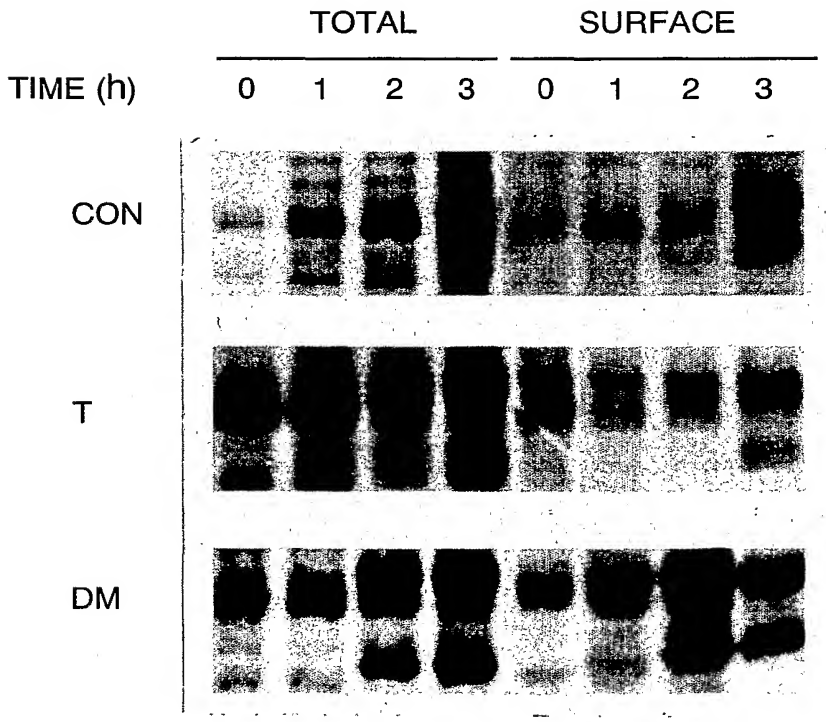
FIG. 1B



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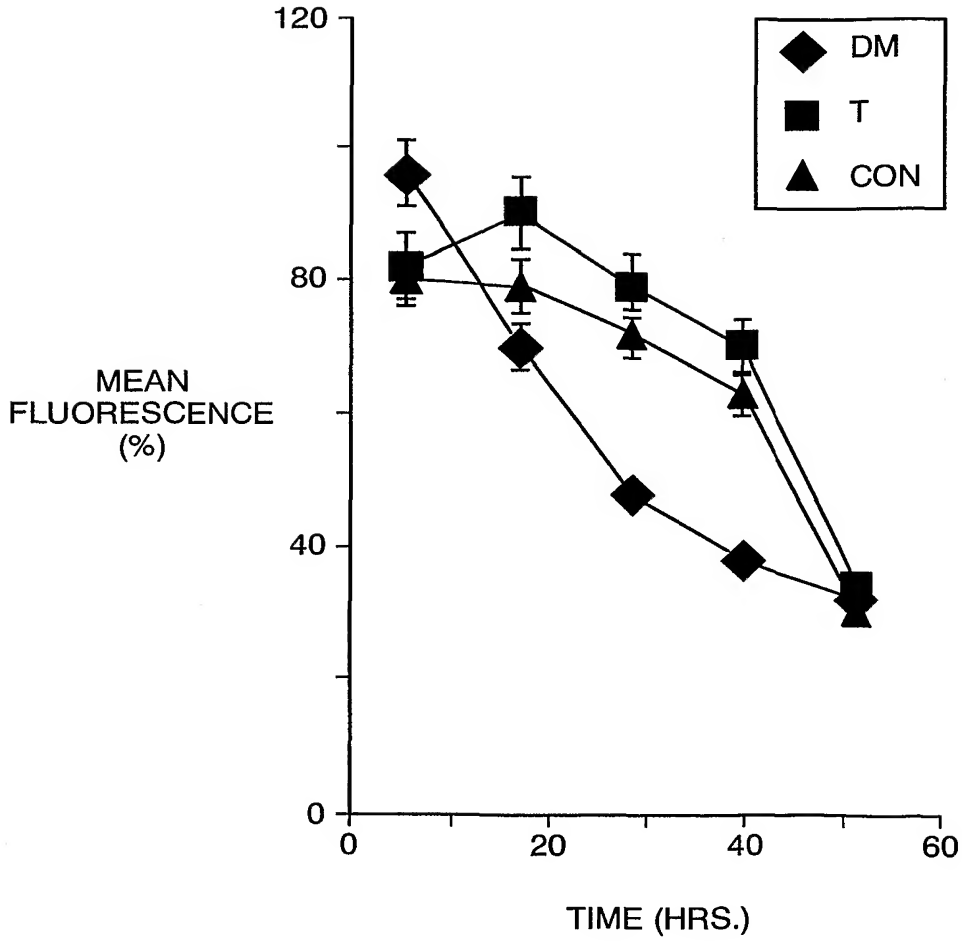

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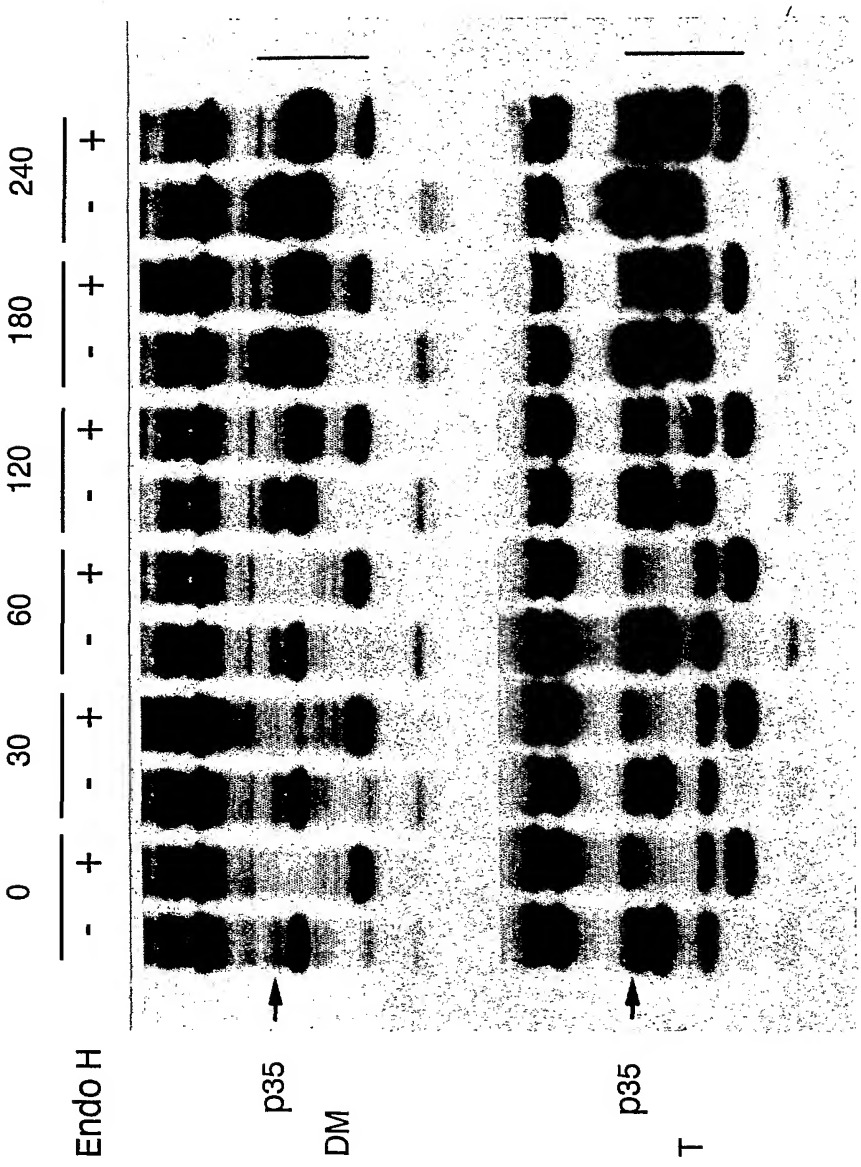

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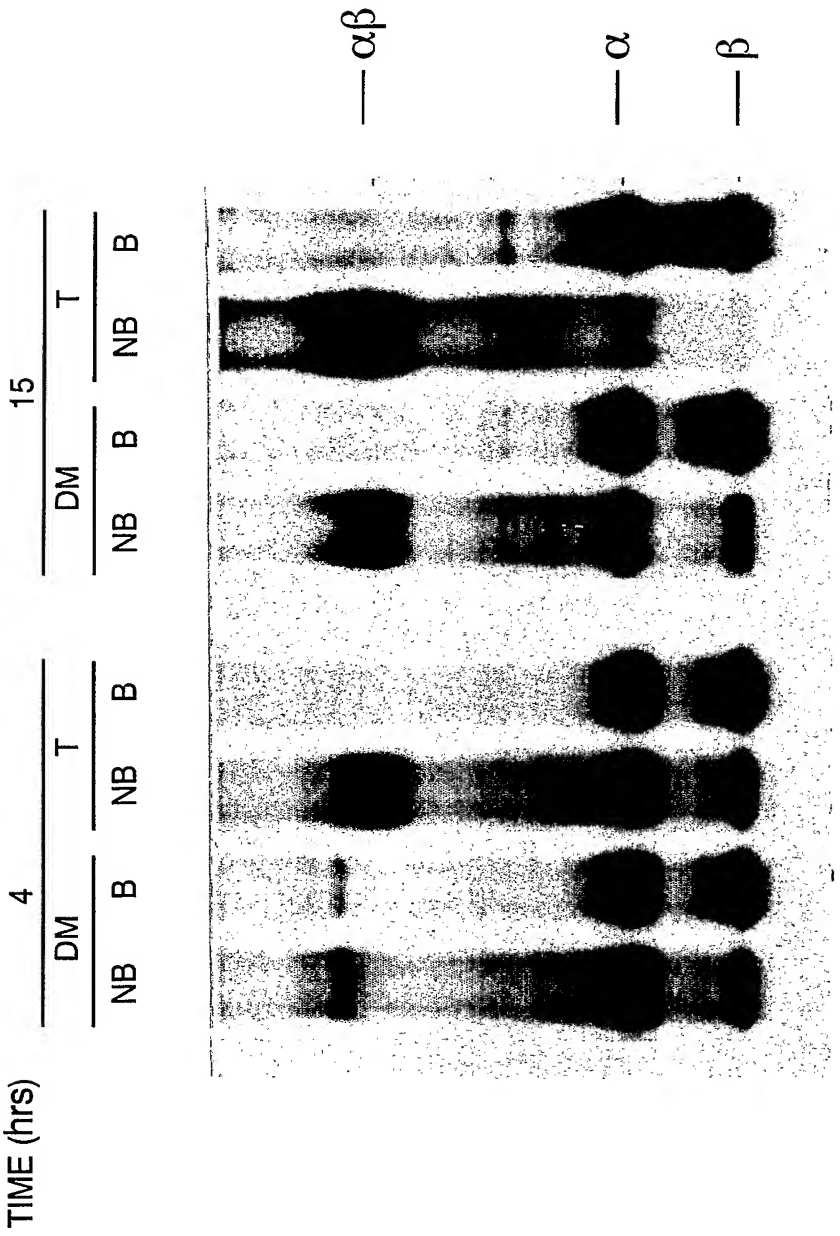
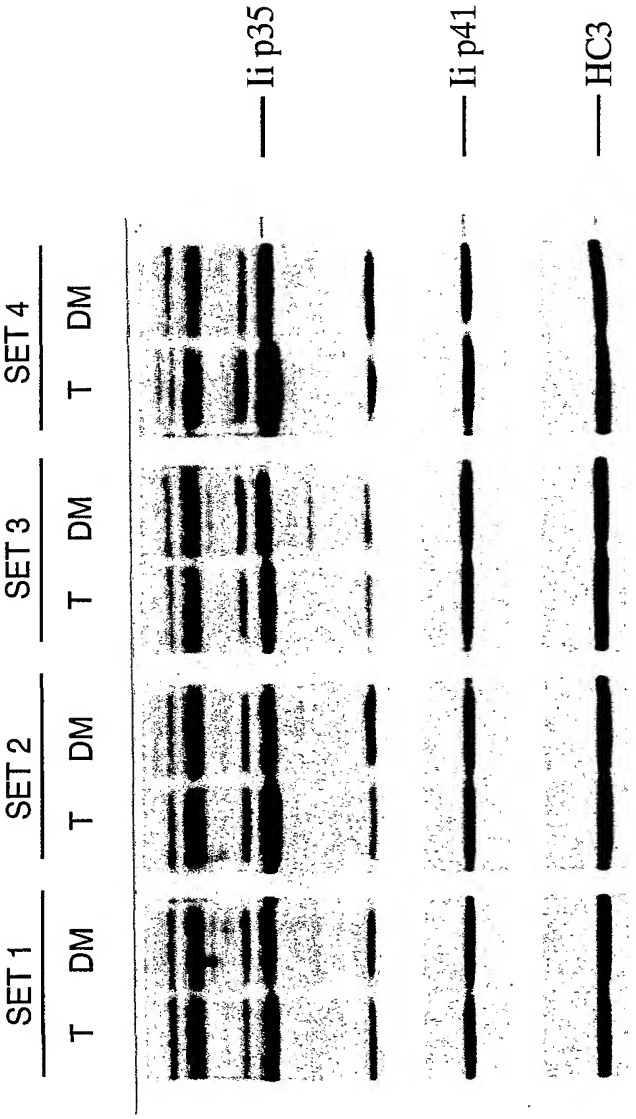


FIG. 5



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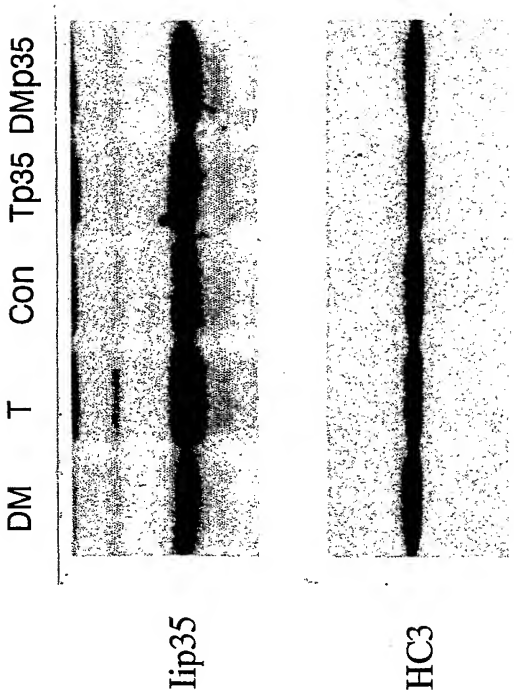

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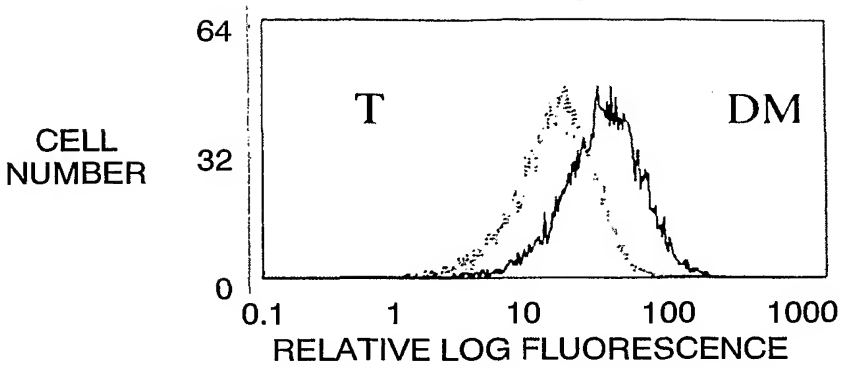

FIG. 8A

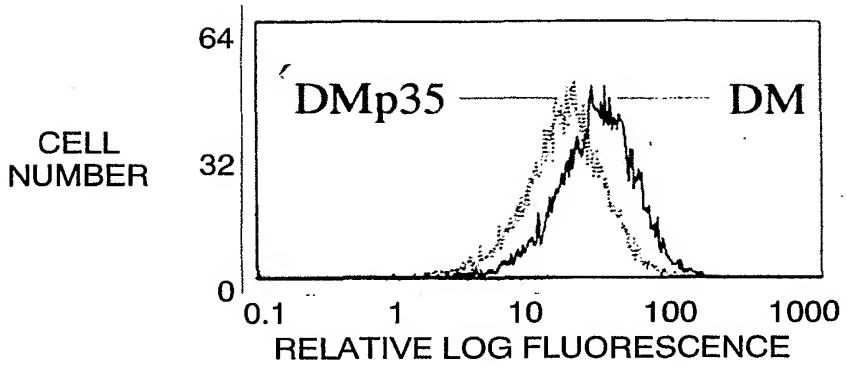

FIG. 8B

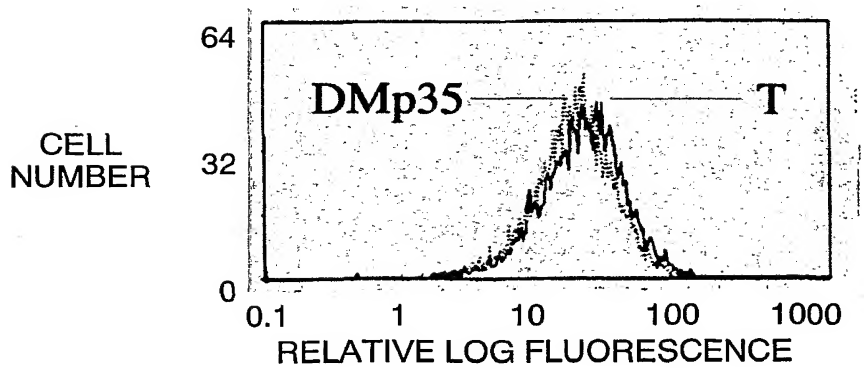

FIG. 8C

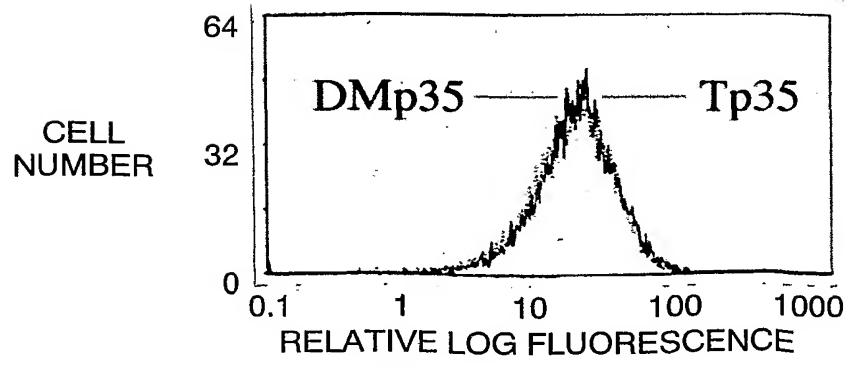

FIG. 8D

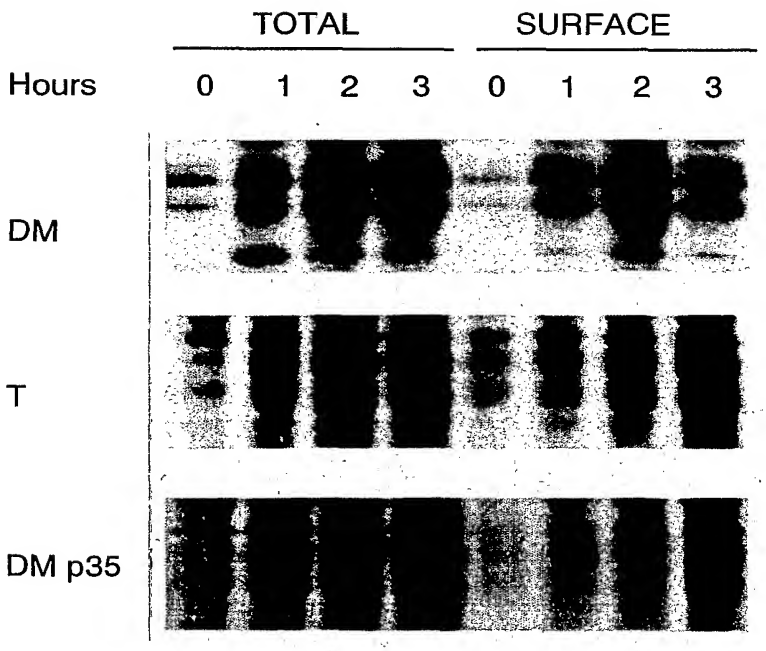

FIG. 9

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Faustman, Denise L.

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